Immunoreactive substance P and neurokinin A in the hypothalamus and anterior pituitary gland of Siberian and Syrian hamsters and of rats

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In this investigation the concentrations of immunoreactive substance P and neurokinin A in the hypothalamus and anterior pituitary of the Siberian hamster were compared with those in the rat and Syrian hamster. The concentrations of immunoreactive neurokinin A in the hypothalamus of Siberian hamsters were significantly higher than those of rats and Syrian hamsters, while male Siberian hamsters had similar amounts of substance P in the hypothalamus to those of male Syrian hamsters, but had higher amounts than those in male rats. However, female Siberian hamsters had significantly higher hypothalamic concentrations of both substance P and neurokinin A than did female Syrian hamsters and rats. In the anterior pituitary glands of Siberian hamsters, concentrations of substance P and neurokinin A were markedly higher than they were in rats and even more so than in Syrian hamsters. Ovariectomy further increased tachykinin concentrations in the anterior pituitary gland of female Siberian hamsters, and this was completely prevented by oestradiol replacement. Female Siberian hamsters kept under conditions of reduced photoperiod had significantly higher tachykinin concentrations in the anterior pituitary than did animals kept under daily photoperiods of 16 h light:8 h dark. The incubation of anterior pituitaries from female Siberian hamsters with a neurokinin A receptor antagonist resulted in a partial blockade of the LH and FSH release in response to LHRH. Thus, the high concentration of tachykinins present in the anterior pituitary of the Siberian hamster may have a local role in modulating the secretion or release of gonadotrophins.

Introduction

There is increasing evidence suggesting that tachykinins have a role in the regulation of the secretion of anterior pituitary hormones (Kato *et al.*, 1976; Vijayan and McCann, 1979; Dees *et al.*, 1985; Debeljuk *et al.*, 1987; Pisera *et al.*, 1991). Both substance P and neurokinin A, and the elongated forms of neurokinin A, neuropeptide K and neuropeptide γ , have been detected in hypothalamic extracts from different species; these substances are also present in much lower amounts in the anterior pituitary gland, except for neuropeptide K, the presence of which has not been detected in this gland (Coslovsky *et al.*, 1984; Brown *et al.*, 1990; Debeljuk *et al.*, 1990a). Substance P and neurokinin A are co-synthesized within a larger polypeptide molecule called preprotachykinin (Maggio, 1988).

Steroid hormones are known to affect the content of substance P and neurokinin A in the rat hypothalamus and also in the rat anterior pituitary (Coslovsky *et al.*, 1984; De Palatis *et al.*, 1985; Brown *et al.*, 1990; Debeljuk *et al.*, 1990b). In particular, oestradiol has a profound influence on the stores of substance P and neurokinin A in the anterior pituitary. In

studies from different laboratories, oestradiol has been shown to depress the concentrations of substance P, neurokinin A and preprotachykinin in the anterior pituitary (Coslovsky *et al.*, 1984; De Palatis *et al.*, 1985; Brown *et al.*, 1990; Debeljuk *et al.*, 1991, 1992a). A decrease in the content of substance P in the anterior pituitary of female monkeys during the oestradiol-induced LH surge has also been reported (Kerdelhué *et al.*, 1993).

Most of the studies have been carried out in rats, and little is known about these effects in other species. During the course of studies on the possible effect of environmental light on the hypothalamo–pituitary axis of the Siberian hamster, we first noticed that the anterior pituitary gland in this species contains concentrations of neurokinin-A-immunoreactive substances that are considerably higher than in other species investigated so far, such as rats, Syrian hamsters or mice. We therefore decided to expand our preliminary study of the concentrations of substances resembling substance P and neurokinin A in the hypothalamus and anterior pituitary of the male and female Siberian hamster by comparing these concentrations with those found in two other widely used laboratory animals – rats and Syrian hamsters.

Since in the rat (Brown *et al.*, 1990; Debeljuk *et al.*, 1992a) and the Syrian hamster (L. Debeljuk *et al.*, unpublished),

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ovariectomy and oestradiol replacement significantly affects the tachykinin concentrations in the anterior pituitary, we also tested the possibility that there may be similar effects in Siberian hamsters.

The Siberian hamster is a well-characterized rodent with respect to its reactions in response to changes in the environmental photoperiod (Hoffmann, 1981; Niklowitz and Hoffmann, 1988). These animals undergo gonadal atrophy and reduced gonadal steroid secretion if placed in an environment with a short daily period of light. In female Siberian hamsters exposed to short daily photoperiods that result in reduced serum oestradiol concentrations, changes in tachykinin concentrations in the anterior pituitary should therefore be qualitatively similar to those observed after ovariectomy. This hypothesis was tested with an additional experiment, in which anterior pituitary tachykinin concentrations were determined in Siberian hamsters under short or long daily photoperiods.

Finally, to test the hypothesis that the high tachykinin concentrations in the anterior pituitary of the Siberian hamster have a role in the release of some of the anterior pituitary hormones, anterior pituitary glands from this species were incubated *in vitro*, in the presence of a potent non-peptide neurokinin A receptor antagonist (Emonds-Alt *et al.*, 1992). We speculated that the blockade of neurokinin A receptors in the anterior pituitary could result in changes in basal or LHRH-induced release of LH and FSH, or in both processes.

Materials and Methods

Animals and treatments

Siberian hamsters (*Phodopus sungorus sungorus*) of both sexes were raised in our vivarium from a colony originated from animals kindly made available by Dr B. Goldman (Storrs, CT). They were maintained on a standard laboratory diet with free access to tap water, under conditions of a controlled temperature (21°C) and a daily photoperiod of 16 h light:8 h dark. This photoperiod is routinely used in our vivarium as 'long day' conditions for photoperiod-sensitive animals such as Siberian and Syrian hamsters. Rats were from the Sprague-Dawley strain and were purchased from Harlan (Indianapolis, IN). They were maintained on a standard laboratory diet with free access to tap water, under conditions of constant temperature and a photoperiod of 12 h light:12 h dark. Syrian hamsters (*Mesocricetus auratus*) were obtained from Sasco Inc (Omaha, NE) and kept under the same environmental conditions as the Siberian hamsters.

The age range of all the animals from the three species used in this experiment was between 3 and 6 months at the time of killing.

Experiment 1

Animals of the three species investigated were killed by decapitation, the skull was quickly opened, and the hypothalamus was cut in rhomboidal fragments according to the following limits: anterior, at the optic chiasma; posterior, just before the mamillary bodies; and lateral, at the lateral sulci. The anterior pituitary gland was exposed, the neurointermediate

lobe discarded, and the anterior pituitary was removed. Both tissues (hypothalamus and anterior pituitary) were weighed and immediately immersed individually in polypropylene tubes containing 0.5 ml of ice-cold 2 mol acetic acid l⁻¹ in water. The tubes were then placed in a bath of boiling water for 10 min, to inactivate the proteolytic enzymes contained in the tissues. The fragments were homogenized by sonication, the homogenates were centrifuged at 3500 g for 15 min, and the supernatants were aspirated, frozen at -70°C and later lyophilized. The concentrations of substance P and neurokinin A in the hypothalamus and anterior pituitaries of the three species were compared.

Experiment 2

A second experiment was carried out in female Siberian hamsters to test their responses to ovariectomy and oestradiol replacement. The animals were ovariectomized by a dorsal approach under light ether anaesthesia. Some of the ovariectomized animals were implanted with capsules approximately 1 cm long that had been filled with oestradiol (Sigma Chemical Co, St Louis, MO), while the other hamsters were implanted with empty Silastic capsules. Eighteen days later the animals were killed by decapitation and the anterior pituitary was removed and processed as described for Expt 1. Ovary-intact, sham-operated, female controls were killed at the same time as the ovariectomized animals, but only females showing vaginal cytology characteristic of dioestrus were chosen for this group.

Experiment 3

Female Siberian hamsters kept under the same 16 h light:8 h dark photoperiod (long days) as described above were compared with a similar group of female animals that were kept in a separate room under a short daily photoperiod (short days) of 6 h light:18 h dark. Two months later both groups of animals were killed by decapitation, but among the Siberian hamsters under long days only those exhibiting vaginal cytology characteristic of dioestrus were killed, and the anterior pituitary glands were exposed, removed and processed as indicated for Expt 1.

Experiment 4

A fourth experiment was carried out using anterior pituitary glands from female Siberian hamsters in dioestrus. The effect of the non-peptide NK₂ receptor antagonist SR48968 (*S-N*-methyl-4-(4-acetyl-amino-4-phenyl piperidino)-2-(3,4-dichlorophenyl)butyl benzamide; Sanofi Recherche, Montpellier) on the release of LH and FSH by anterior pituitaries from Siberian hamsters was tested *in vitro*. This receptor antagonist blocks NK₂ receptors, which bind to neurokinin A. SR48968 was dissolved in a small volume of ethanol and then diluted 1:10 with incubation medium (TC-199 – see below). Aliquots of this solution were added to the incubation medium. Siberian hamster anterior pituitary glands were removed and placed in glass tubes containing 0.5 ml synthetic medium TC-199 with Hepes (Sigma Chemical Co),

plus 0.1% BSA (Sigma Chemical Co) and 1.42 mg bacitracin 100 ml (Fluka Chemical Corp, Ronkonkoma, NY). One anterior pituitary was incubated in each tube. The tubes containing the glands were placed in a Dubnoff metabolic shaker at 37°C at a rate of 50 strokes per min, and were constantly gassed with carbogen (Puritan-Bennett Corp, Lenexa, KS). After 1 h, the medium was discarded and new medium was dispensed into each tube. The new medium contained: (1) vehicle (control group); (2) 10 µg SR48968 ml⁻¹; (3) 50 µg SR48968 ml⁻¹; (4) 40 ng LHRH ml⁻¹; or (5) 40 ng LHRH ml⁻¹ plus 50 µg SR48968 ml⁻¹. The incubation proceeded for 3 h, after which the tubes were removed and the media were aspirated and kept frozen at -20°C until assayed for LH and FSH.

Assays

Each hypothalamic extract was resuspended in 0.4 ml of 0.1 mol acetic acid l⁻¹ just before the assay. Aliquots of 10–20 µl were dispensed in each assay tube, in duplicates. The anterior pituitary extracts were resuspended in 1 ml of assay buffer (0.5% BSA-PBS, plus 20 µmol bacitracin l⁻¹; Fluka), thoroughly mixed in a Vortex mixer and centrifuged at 3500 g for 10 min, to eliminate insoluble particles. From the supernatant, 0.5 ml aliquots were dispensed into two tubes, one for neurokinin A and the other for substance P determinations, respectively.

Neurokinin A was determined using a radioimmunoassay previously described (Debeljuk *et al.*, 1990a, 1991), with some modifications; these mainly regarded the separation of bound and free tracer, for which the second antibody method is now used instead of charcoal-dextran. This assay detects neurokinin A, and also the neurokinin A contained in its two elongated forms neuropeptide K and neuropeptide γ. The antiserum shows between 40 and 60% crossreactivity with neuropeptides K and γ, and this was confirmed by the presence of a peak corresponding to the elution of neuropeptide K in extracts of rat hypothalami purified by HPLC (Villanúa *et al.*, 1992). However, rat anterior pituitary extracts do not seem to contain measurable quantities of neuropeptide K, since no peak corresponding to this peptide was found in anterior pituitary extracts purified by HPLC (Brown *et al.*, 1990; Debeljuk *et al.*, 1991). Synthetic neurokinin A, Bolton-Hunter-labelled with ¹²⁵I (Amersham Corp, Arlington Heights, IL), was used as a tracer. Dose-response curves of rat and Syrian hamster hypothalamic and anterior pituitary extracts and synthetic neurokinin A had been reported by Debeljuk *et al.* (1990a, 1992b). Synthetic neurokinin A (Cambridge Research Biochemicals Inc, Wilmington, DE) was used as a standard preparation. Intra-assay and interassay coefficients of variation were 7.8 and 11.4%, respectively. In the present investigation dose-response curves of synthetic neurokinin A, and of hypothalamic and anterior pituitary extracts from Siberian hamsters were studied and compared. In both cases the dose-response curves were similar (Fig. 1).

Substance P was determined in hypothalamic and anterior pituitary extracts of the three species investigated using a double-antibody radioimmunoassay already described (Debeljuk *et al.*, 1992b). Synthetic substance P (Cambridge

Fig. 1. Dose-response curves of synthetic neurokinin A (□) and (a) hypothalamic and (b) anterior pituitary (■) extracts from Siberian hamsters. The doses of hypothalamic extracts were 1, 2.5, 5, 10, 20 and 40 µl (1 hypothalamus per 400 µl), and the doses of anterior pituitary extracts were 7.81, 15.62, 31.25, 62.5, 125, 250 and 500 µl (1 anterior pituitary per 250 µl).

Research Biochemicals Inc) was used as the standard preparation, and substance P that had been Bolton-Hunter-labelled with ¹²⁵I (Amersham Corp) was used as the tracer. Dose-response curves of synthetic substance P were parallel to dose-response curves of rat and Syrian hamster hypothalamic extracts, as described by Debeljuk *et al.* (1992b). Intra-assay and interassay coefficients of variation were 1.5 and 10.6%, respectively. In the present investigation, dose-response curves of synthetic substance P (Cambridge Research Biochemicals Inc) and hypothalamic and anterior pituitary extracts from Siberian hamsters were run in parallel, and the curves were similar (Fig. 2).

All the neurokinin A and substance P determinations of hypothalamic and anterior pituitary extracts from the three species under study were performed in a single assay, except for the determination of substance P in anterior pituitary extracts of Syrian hamsters, which were performed in a separate assay. The experiments with ovariectomized and oestradiol-treated Siberian hamsters and with animals under short and long photoperiods were performed some time after these first experiments; the samples were therefore run in a separate, later assay.

Fig. 2. Dose-response curves of synthetic substance P ($\square$) and (a) hypothalamic ($\blacksquare$) and (b) anterior pituitary ($\blacksquare$) extracts from Syrian hamsters. The doses of hypothalamic extracts were 12.5, 25, 50, 125, 250, 375 and 500 μ l (1 hypothalamus per 500 μ l), and the doses of anterior pituitary extracts were 10, 25, 50, 100, 250 and 500 μ l (1 anterior pituitary per 250 μ l).

The results were expressed as pg of synthetic neurokinin A or substance P mg^{-1} wet tissue. One ng of neurokinin A is equivalent to $0.882 \text{ pmol l}^{-1}$ and 1 ng of substance P is equivalent to $0.7425 \text{ pmol l}^{-1}$.

In the incubation media, LH and FSH were determined by means of double-antibody radioimmunoassays using kits distributed by the National Hormone and Pituitary Program, NIH, by courtesy of A. F. Parlow and S. Raiti.

Statistical analysis

The significance of the differences between groups was determined by analysis of variance followed by Dunnett's test using a computer program (STATVIEW 512) for the Macintosh. Since the values of the SEs of the neurokinin A and substance P values in the anterior pituitary extracts from Syrian hamsters were markedly higher than those from rats and Syrian hamsters, all the anterior pituitary data were analysed after logarithmic transformation. In Expt 3 Student's *t* test was used.

Fig. 3. Neurokinin A concentrations (pg mg^{-1} wet tissue) in hypothalamic extracts of Syrian hamsters ($\square$; $n = 13$ males and 15 females), Syrian hamsters ($\blacksquare$; $n = 14$ males and 10 females), and rats ($\square$; $n = 10$ males and 10 females). * $P < 0.05$ compared with Syrian hamsters; ^a $P < 0.05$ compared with Syrian hamsters.

Results

Experiment 1

Male Syrian hamsters had significantly higher hypothalamic concentrations of immunoreactive neurokinin A than did Syrian hamsters and rats, and Syrian hamster hypothalami had significantly higher neurokinin A concentrations than did rat hypothalami ($P < 0.05$) (Fig. 3). Since the hypothalamic fragments from Syrian hamsters were smaller than those of Syrian hamsters, a useful comparison is the content of neurokinin A in the whole hypothalamus rather than the concentration per mg of tissue; if this is calculated, the values are similar for Syrian and Syrian hamsters (2559.4 ± 112.4 versus 2694.3 ± 290.6 pg, respectively), but still significantly higher ($P < 0.05$) than in the rat (1235.7 ± 41.6 pg). Female Syrian hamsters also had significantly higher hypothalamic neurokinin A concentrations than did female Syrian hamsters and rats, but in the last two species these concentrations were similar. The amount of neurokinin A in the whole hypothalamus was not significantly different in the three species, although in the rats (2246.3 ± 106.2 pg) the values were lower than in the Syrian (2450.1 ± 108.4 pg) or the Syrian (2337.5 ± 192.6 pg) hamster.

Hypothalamic concentrations of immunoreactive substance P in male Syrian and Syrian hamsters were not significantly different, but they were higher than in male rats ($P < 0.05$) (Fig. 4). If the results are expressed as the content in the whole hypothalamus, since the hypothalamus of the Syrian hamster is smaller than that of the other two animal species, the content of substance P in the hypothalamus of the Syrian hamster (2986.6 ± 220.8 pg) is higher than that in the Syrian hamster (2166.0 ± 122 pg), and both values are also significantly higher ($P < 0.05$) than that of rats (1748.0 ± 78.7 pg). Female Syrian hamsters had significantly higher hypothalamic concentrations of substance P than did female Syrian hamsters and rats, while the last two species had similar hypothalamic concentrations of substance P. The content of substance P in the whole hypothalamus was 2292.9 ± 195.0 pg for Syrian hamsters, 1915.2 ± 131.7 pg for Syrian hamsters, and 1722.7 ± 107.1 pg

Fig. 4. Substance P concentrations (pg mg^{-1} wet tissue) in the hypothalamus of Siberian hamsters ($\square$; $n = 13$ males and 15 females), Syrian hamsters ($\blacksquare$; $n = 14$ males and 10 females), and rats ($\square$; $n = 10$ males and 10 females). $^*P < 0.05$ compared with Syrian hamsters; $^aP < 0.05$ compared with Siberian hamsters.

Fig. 5. Neurokinin A concentrations (pg mg^{-1} wet tissue) in the anterior pituitary of Siberian hamsters ($\square$; $n = 11$ males and 10 females), Syrian hamsters ($\blacksquare$; $n = 9$ males and 10 females), and rats ($\square$; $n = 10$ males and 12 females). $^*P < 0.05$ compared with Syrian hamsters; $^aP < 0.05$ compared with Siberian hamsters.

for rats, which is significantly lower ($P < 0.05$) than in both types of hamster.

In the anterior pituitary gland, neurokinin A concentrations were markedly higher in male Siberian hamsters than in male rats and Syrian hamsters ($P < 0.05$) (Fig. 5), and in the rat the concentration of neurokinin A in the anterior pituitary was significantly higher than that in Syrian hamsters ($P < 0.05$). In general, concentrations of neurokinin A in the anterior pituitary were lower in females than in the respective males, and again, in females, the highest concentrations were found in Siberian hamsters, followed by rats and Syrian hamsters, in that order ($P < 0.05$).

Concentrations of substance P were significantly higher in the anterior pituitary glands of male and female Siberian hamsters than in rats and Syrian hamsters, but in both male and female rats these concentrations were significantly higher than in Syrian hamsters ($P < 0.05$) (Fig. 6). In male Siberian hamsters, the concentration of substance P in the anterior pituitary was

Fig. 6. Substance P concentrations (pg mg^{-1} wet tissue) in the anterior pituitary of Siberian hamsters ($\square$; $n = 11$ males and 10 females), Syrian hamsters ($\blacksquare$; $n = 9$ males and 10 females), and rats ($\square$; $n = 10$ males and 12 females). $^*P < 0.05$ compared with Syrian hamsters; $^aP < 0.05$ compared with Siberian hamsters.

Fig. 7. Neurokinin A concentrations (pg mg^{-1} wet tissue) in the anterior pituitary gland of intact, ovariectomized (OVX), and ovariectomized, oestrogen-treated (OVX + E) Siberian hamsters ($n = 11$ –12 per group). $^*P < 0.05$ compared with Siberian hamsters; $^aP < 0.05$ compared with intact or ovariectomized Siberian hamsters.

significantly higher than in females of the same species by a factor of 2.74, while the amount of neurokinin A in this gland was 1.7 times higher in males than in females.

Experiment 2

In female Siberian hamsters, ovariectomy was followed by a marked increase in the concentration of neurokinin A and an even greater increase in the concentration of substance P in the anterior pituitary compared with intact females ($P < 0.05$) (Figs 7 and 8). The oestradiol replacement in the form of implants completely prevented this increase.

Experiment 3

Siberian hamsters kept under short days had significantly higher concentrations of neurokinin A and substance P in the anterior pituitary than in similar animals kept under long days ($P < 0.01$) (Fig. 9).

Fig. 8. Substance P concentrations (pg mg^{-1} wet tissue) in the anterior pituitary gland of intact, ovariectomized (OVX), and ovariectomized, oestrogen-treated (OVX + E) Siberian hamsters ($n = 11$ – 12 per group). * $P < 0.05$ compared with intact Siberian hamsters; ^a $P < 0.05$ compared with ovariectomized Siberian hamsters.

Fig. 9. (a) Neurokinin A and (b) substance P concentrations (pg mg^{-1} wet tissue) in the anterior pituitary of female Siberian hamsters kept under short (SD; $n = 8$) or long (LD; $n = 10$) daily photoperiods. * $P < 0.01$ compared with LD.

Experiment 4

LH concentrations in media from anterior pituitaries incubated with 10 or 50 $\mu\text{g SR48968 ml}^{-1}$ were not significantly different from those of the control group (Fig. 10), but 50 $\mu\text{g SR48968 ml}^{-1}$ stimulated the release of FSH into the medium ($P < 0.05$). LHRH induced significant increases in the release of LH and FSH into the medium ($P < 0.05$), and the group of anterior pituitaries incubated with LHRH plus SR48968 had significantly higher LH and FSH concentrations ($P < 0.05$) than did the control group, but these concentrations were significantly lower than those in the LHRH group ($P < 0.05$).

Discussion

The Siberian (also called Djungarian) hamster is a rodent whose pituitary and gonadal functions are greatly influenced by the environmental photoperiod (Hoffmann, 1981; Yellon and

Fig. 10. (a) LH and (b) FSH concentrations in the medium from Siberian hamster anterior pituitaries incubated with vehicle (control), 10 $\mu\text{g SR48968 ml}^{-1}$ (SR 10), 50 $\mu\text{g SR48968 ml}^{-1}$ (SR 50), LHRH, or LHRH plus 50 $\mu\text{g SR48968 ml}^{-1}$ (LHRH + SR 50) ($n = 9$ – 10 tubes per group). * $P < 0.05$ compared with control; ^a $P < 0.05$ compared with LHRH.

Goldman, 1987; Niklowitz and Hoffmann, 1988). This species has been studied quite extensively and there is considerable and increasing interest in its neuroendocrine and gonadal functions. However, to our knowledge, this species has never been studied with regard to the content of tachykinins in its different organs. From the studies reported here it is evident that the hypothalamus of both male and female Siberian hamsters contains higher concentrations of neurokinin-A-immunoreactive substances (these may include neuropeptide K and neuropeptide γ) than does that of the Syrian hamster and the rat, and also contains more substance P than does the hypothalamus of rats and female Syrian hamsters (male Siberian and Syrian hamsters had similar hypothalamic concentrations of substance P).

In the anterior pituitary gland the differences in tachykinin concentrations were particularly marked in Siberian hamsters compared with the Syrian hamsters or rats. They were also much higher than the tachykinin concentrations in the anterior pituitary of mice (L. Debeljuk *et al.*, unpublished). These high concentrations of neurokinin A and substance P in the anterior pituitary of the Siberian hamsters have been repeatedly confirmed in additional experiments performed in our laboratory with the same species (L. Debeljuk *et al.*, unpublished). Thus, in two additional experiments, neurokinin A in the anterior pituitary of male Siberian hamsters was $214.2 \pm 15.58 \text{ pg mg}^{-1}$ wet tissue ($n = 19$) and 261.8 ± 20.84 ($n = 12$), while substance P values in one experiment were 400.2 ± 48.14

($n = 13$). The differences in the concentrations of neurokinin A and substance P in the anterior pituitary between the Siberian and the Syrian hamster range from a factor of 16 to 31. The rat had higher concentrations of neurokinin A and substance P in the anterior pituitary than did the Syrian hamster, but still 3–7 times less than the Siberian hamster.

Another interesting finding in the anterior pituitary gland of these three species is that the concentrations of substance P generally showed a tendency to be higher than those of neurokinin A, while in the hypothalamus the concentration of neurokinin A was slightly higher than that of substance P or, in some cases, similar. However, these data do not reveal why the tachykinins in the anterior pituitary of the Siberian hamster are so much higher than those in other species studied so far.

Recent studies have revealed that substance P in the human and rat anterior pituitary gland is present in subsets of somatotrophs and thyrotrophs (Roth and Krause, 1990; Brown *et al.*, 1991), although earlier studies using electron microscopy had revealed the presence of substance P in the mammotrophs and gonadotrophs (Morel *et al.*, 1982). No similar study has been reported so far on the presence of neurokinin A, but it is highly likely that neurokinin A is contained in the cells that contain substance P, as these two tachykinins are largely co-synthesized within the precursor molecules preprotachykinins β and γ (Maggio, 1988). It is therefore possible that in Siberian hamsters the high concentrations of tachykinins in the anterior pituitary gland are either due to a higher amount of these peptides present in the somatotrophs and thyrotrophs (i.e. the same cells that were shown to contain them in the anterior pituitary of the rat), or that in the anterior pituitary of the Siberian hamster some other cells could contain stores of substance P and neurokinin A. Another point to be considered is that the large tachykinin stores in the anterior pituitary of the Siberian hamster may come mainly from autochthonous biosynthesis in the gland, or the tachykinins may be released from the neural terminals in the median eminence and then taken up from the portal blood by some anterior pituitary cells. Since the hypothalamus of the Siberian hamsters contains only moderately higher tachykinin concentrations than that of rats or Syrian hamsters, while the anterior pituitary is many times richer in both tachykinins than in the other two species, it seems that a higher rate of tachykinin synthesis in the anterior pituitary may be the most likely explanation.

The possible physiological significance of the high tachykinin concentrations in the anterior pituitary of this species is another point that should be clarified. There are several reports suggesting that tachykinins influence the release of LH and prolactin (Battmann *et al.*, 1991; Pisera *et al.*, 1991; Kalra *et al.*, 1992), some of which imply a direct action at the anterior pituitary level (Kerdellhué *et al.*, 1979; Shamgochian and Leeman, 1992). An additional possibility is that tachykinins may have a paracrine intrapituitary role. The anterior pituitary contains the biosynthetic machinery to produce tachykinins (Jonassen *et al.*, 1987; Brown *et al.*, 1990), and the presence of specific receptors to substance P in gonadotrophs and mammotrophs has been reported by Larsen *et al.* (1992). This finding indicates that tachykinins are produced in some somatotrophs and thyrotrophs, that they are probably released and may act locally on other anterior pituitary cells such as

gonadotrophs and mammotrophs. Our experiment *in vitro* to test the effect of a potent NK₂ receptor antagonist on the anterior pituitary suggests an intrapituitary role of neurokinin A, neuropeptide K and neuropeptide γ (which bind to NK₂ receptors) on LH and FSH release. The antagonist used in this investigation partially blocked the response to LHRH, and this suggests that in the anterior pituitary of Siberian hamsters, NK₂ receptors mediate stimulatory effects of these tachykinins on gonadotrophin release. The effect of the highest dose of the antagonist stimulating the basal release of FSH remains to be investigated further. We have recently observed in incubations of rat hemipituitaries that the same NK₂ receptor antagonist also inhibits the response to LHRH and induces a significant decrease in prolactin release (L. Debeljuk and A. Bartke, unpublished), which suggests that intrapituitary tachykinins stimulate prolactin release as well.

The effects of ovariectomy and oestradiol replacement in the Siberian hamster are similar to those observed in rats (Coslovsky *et al.*, 1984; De Palatis *et al.*, 1985; Debeljuk *et al.*, 1992a). In this respect, the increase in substance P in the anterior pituitary after ovariectomy was particularly striking, since substance P reached concentrations that were only slightly lower than those observed in the hypothalamus of the same species. As revealed in studies in other species, the concentrations of tachykinins in the hypothalamus were much higher than in the anterior pituitary gland (Brown *et al.*, 1990; Debeljuk *et al.*, 1990b, 1991) and as in rats oestradiol replacement completely prevented these changes. The effects of ovariectomy and oestradiol replacement on the concentrations of substance P and neurokinin A in the anterior pituitary suggest that sexual dimorphism in the concentration of these tachykinins is due to an inhibitory influence of the ovaries, and that oestradiol is the likely mediator of this effect.

It has been shown that oestradiol decreases the secretion by the somatotrophs and the concentration of circulating GH (Ho *et al.*, 1988), and also decreases the percentage of somatotrophs with substance P immunoreactivity (Roth and Krause, 1990). Oestradiol may induce somatotrophs to differentiate to mammotrophs (Strattman *et al.*, 1974). It therefore seems likely that the decrease in the concentrations of substance P and neurokinin A in the anterior pituitary by oestradiol may be exerted mainly through an effect on the somatotrophs. Similar to the results obtained after ovariectomy, exposure to a short day photoperiod also resulted in increases of tachykinin concentrations in the anterior pituitary gland. The mechanism of this effect is probably the same as for the ovariectomy: a decreased concentration of oestrogen in the circulation. As in the case of ovariectomy, the increase in the concentration of substance P in the anterior pituitary was higher than that of neurokinin A (although the values for substance P were more dispersed, as indicated by a greater SE).

In summary, the results of this investigation show that the anterior pituitary gland of the Siberian hamsters contain much higher concentrations of tachykinins than do those in other common laboratory animals, and these anterior pituitary stores are significantly influenced by ovariectomy, exposure to short day photoperiod and oestradiol administration. Tachykinins might have an intrapituitary role as facilitatory modulators of gonadotrophin release.

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