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Neurokinin 3 Receptor Antagonism Reveals Roles for Neurokinin B in the Regulation of Gonadotropin Secretion and Hot Flashes in Postmenopausal Women

Karolina Skorupskaite[a] Jyothis T. George[b, c] Johannes D. Veldhuis[d]
Robert P. Millar[e, f] Richard A. Anderson[a]

[a]MRC Centre for Reproductive Health, The Queen's Medical Research Institute, University of Edinburgh, Edinburgh, [b]Warwick Medical School, Coventry, and [c]Boehringer Ingelheim, Bracknell, UK; [d]Endocrine Research Unit, Center for Translational Science Activities, Mayo Clinic, Rochester, MN, USA; [e]Centre for Neuroendocrinology and Mammal Research Institute, University of Pretoria, Pretoria, and [f]Institute for Infectious Diseases and Molecular Medicine, University of Cape Town, Cape Town, South Africa

Keywords
Neurokinin B · Kisspeptin · Gonadotropin-releasing hormone · Menopause · Luteinising hormone pulsatility · Hot flashes

Abstract
Objectives: Neurokinin B (NKB) and kisspeptin are obligate for normal gonadotropin secretion, and links between gonadotropin-releasing hormone (GnRH) pulsatility and vasomotor symptoms have been proposed. Using a selective NKB receptor (NK3R) antagonist, the role of NKB in the hypergonadotropic state in menopausal women was explored. ***Methods:*** Eleven postmenopausal women were administered the NK3R antagonist MLE4901 at 40 mg twice daily orally for 7 days. Ten-minute blood sampling for 8 h was performed before and on the last day of NK3R antagonist treatment for luteinising hormone (LH) pulsatility analysis with kisspeptin-10 (0.3 µg/kg i.v. bolus) administered at 6 h on both days. Hot flash frequency and severity were self-reported for 7 days before and during NK3R antagonist administration. ***Results:*** LH fell from 29.3 ± 4.1 to 24.4 ± 3.8 IU/L ($p < 0.05$) after 7 days of NK3R antagonist treatment, with no change in follicle-stimulating hormone (FSH). Basal (non-pulsatile) LH secretion was reduced (549.0 ± 70.8 vs. 366.1 ± 92.1 IU/L/6 h, $p = 0.006$), and while the LH pulse frequency did not change in the group as a whole (from 0.8 ± 0.1 to 0.7 ± 0.1 pulses/h, ns), it did fall in the 8 women with hot flashes (from 1.0 ± 0.1 to 0.7 ± 0.1 pulses/h, $p < 0.05$). These women also reported a reduction in hot flash frequency (from 3.4 ± 1.2 to 1.0 ± 0.6 hot flashes/day, $p = 0.008$) whilst taking the NK3R antagonist. Kisspeptin-10 did not affect LH secretion with or without the NK3R antagonist. ***Conclusions:*** The administration of an NK3R antagonist indicates a role for NKB in the regulation of LH/GnRH in postmenopausal women, whereas the lack of response to kisspeptin may reflect the hypo-oestrogenic state. These data support a link of LH/GnRH pulsatility and vasomotor symptoms with NK3R antagonism as a potential therapeutic approach.

Introduction

Recent years have seen substantial increases in understanding of the regulation of the secretion of gonadotropin-releasing hormone (GnRH), and thus of the hypothalamic-pituitary-gonadal axis, following the discovery of

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E-Mail karger@karger.com
www.karger.com/nen

Karolina Skorupskaite
MRC Centre for Reproductive Health, The Queen's Medical Research Institute
University of Edinburgh, 47 Little France Crescent
Edinburgh EH16 4TJ (UK)
E-Mail skorupskaite@doctors.org.uk

the involvement of the hypothalamic neuropeptides kisspeptin and neurokinin B (NKB) and their receptors [1–4]. Several studies have shown stimulatory effects of kisspeptin on luteinising hormone (LH) secretion in men and women, the response in healthy women being variable and dependent on the sex steroid milieu across different phases of the menstrual cycle [5, 6] with a direct positive correlation with oestradiol concentrations [7, 8]. Administration of NKB did not affect LH secretion in men or women [9]. Although both kisspeptin and NKB are co-expressed in some hypothalamic neurones [10] which also co-express dynorphin and are termed "KNDy neurones," NKB action appears to be upstream of kisspeptin, as patients with inactivating mutations of NKB and its cognate receptor show diminished LH pulsatility, which is restored by kisspeptin-10 infusion [11]. A recent study of the effect of the neurokinin 3 receptor (NK3R) antagonist MLE4901 (previously termed AZD4901) in women with polycystic ovary syndrome (PCOS) showed some suppression of LH secretion [12], also demonstrated in normal women administered exogenous oestrogen [8].

The only study in which kisspeptin-10 was administered to oestrogen-deficient postmenopausal women showed a rather limited LH response to both kisspeptin-10 and GnRH injection compared to women in the follicular phase or those taking hormonal contraceptives [6]. This suggests that endogenous kisspeptin might already be operating at its maximal capacity to increase GnRH pulsatility and subsequently LH secretion in response to the loss of ovarian negative feedback in postmenopausal women. In keeping with this, hypothalamic expression of *KISS1* and *TAC3* mRNA (encoding kisspeptin and NKB, respectively) is upregulated, and kisspeptin and NKB neurones are hypertrophied in the infundibular nucleus of postmenopausal compared to premenopausal women [13–15]. Similarly, the expression of those neuropeptides is increased in ovariectomised rodents, ewes, and monkeys but restored with oestrogen replacement [16, 17].

In ovariectomised ewes and castrated monkeys, selective blockade of NK3R decreased the frequency of LH pulses (indicative of pulsatile GnRH release), and similar effects were found in women with PCOS, also characterised by a higher LH pulse frequency [12, 18, 19]. However, the inhibitory effect of such blockade on the NKB/kisspeptin/GnRH pathway on high LH secretion in healthy women, such as postmenopausally, has not been explored. Manipulation of high LH/GnRH pulsatility in postmenopausal women may open new paradigms in the management of menopausal hot flashes, since functional links between pathways driving GnRH pulsatility and vasomotor symptoms have been proposed [20]. Synchronisation of LH pulses with hot flashes in women has been demonstrated [21, 22], and administration of exogenous NKB induced hot flash-like symptoms in healthy premenopausal women [23]. Using a selective NK3R antagonist, the role of NKB in the regulation of the hypergonadotropic state in menopausal women and its interaction with kisspeptin was therefore investigated.

Subjects and Methods

Participants

Eleven healthy postmenopausal women aged 46–62 years (2–20 years since natural menopause) were recruited to this study. All subjects provided informed written consent and the study received approval from the Lothian Research Ethics Committee (Ref. No. 09/S1101/67). The subjects were on no hormonal replacement therapy and not taking any preparations for hot flashes. As the primary outcome was analysis of LH secretion, the presence or frequency of hot flashes was not an inclusion criterion, and was reported in 8 of the subjects. Full blood counts, renal function, electrolytes, liver function, and electrocardiograms were within normal limits.

Study Drugs

The specific NK3R antagonist MLE4901 was administered orally at 40 mg twice daily. Human kisspeptin-10 was custom synthesised under GMP standards (Bachem GmBH, Weil am Rhein, Germany); 1 mg kisspeptin-10 was dissolved in 5 mL sterile 0.9% saline immediately before administration as an intravenous bolus of 0.3 μg/kg.

Protocol

A schematic representation of the protocol is shown in Figure 1. The subjects were administered the NK3R antagonist for 7 days. Peripheral venous blood was sampled for spot LH and follicle-stimulating hormone (FSH) 24 h before treatment and after the NK3R antagonist had been administered for 1, 3, 5, and 7 days. Once-daily blood sampling was performed in the morning; during treatment, this was immediately prior to the next dose of NK3R antagonist, i.e., 12 h after the previous dose.

On the pre-treatment day and on the last day of NK3R antagonist administration the subjects attended the clinical research facility for 8 h. All visits commenced between 08:00 and 09:00 h. The last dose of NK3R antagonist was administered, following which blood samples were collected via an indwelling intravenous cannula at 10-min intervals for 6 h for the assessment of LH pulsatility. Kisspeptin-10 was administered after 6 h, with further blood sampling for 2 h every 10 min for LH and hourly for FSH.

The subjects were asked to self-report any flashing symptoms (e.g., heat and sweating) and night-time awakenings, including their frequency and perceived severity (1 "mild" = heat sensation and no sweating; 2 "moderate" = heat and sweating, not causing disruption to activity; 3 "severe" = heat and sweating, disrupting activity), by using daily diaries for a week prior to the study visits and whilst on NK3R antagonist treatment. The subjects were en-

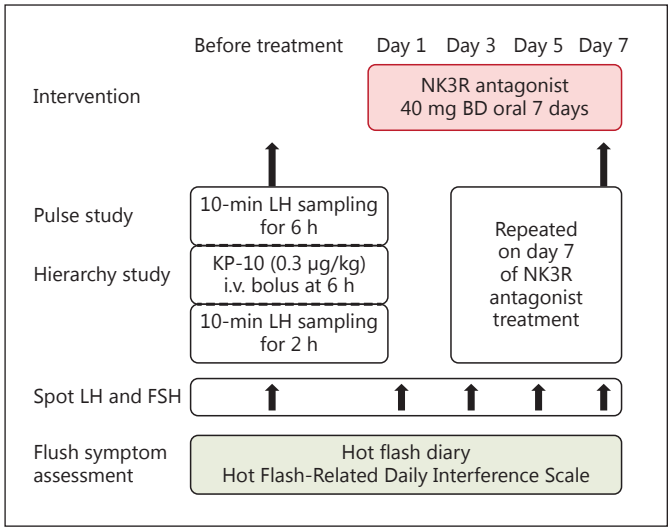

Fig. 1. Study protocol. Eleven healthy postmenopausal women were administered the neurokinin 3 receptor (NK3R) antagonist MLE4901 orally for 7 days. Luteinising hormone (LH) and follicle-stimulating hormone (FSH) were measured throughout the study. LH pulsatility was assessed during 10-min blood sampling for 6 h on the day before and on the last day of NK3R antagonist treatment. Kisspeptin (KP)-10 was administered as an intravenous bolus at 6 h, with further frequent blood sampling for 2 h. The women self-reported the frequency and severity of hot flashes and the interference of those flashes with daily activities throughout the study.

couraged to record the flash and its severity as it occurred to minimise retrospective monitoring. The subjective Hot Flash-Related Daily Interference Scale (HFRDIS) [24], covering the preceding 7 days, was completed by the subjects on the last day before treatment and on completion of the NK3R antagonist treatment.

Safety blood samples, including for the assessment of full blood counts, renal function, electrolytes, and liver function, were drawn before commencing the NK3R antagonist treatment, 2–3 weeks after the treatment had finished, and at the end of each 8-h visit for frequent blood sampling. The hormone assays, blinded LH pulsatility measurements, and safety blood tests were performed as previously described [8, 25].

Statistical Analysis

Mean LH and FSH concentrations over time were compared using 1-way ANOVA followed by Bonferroni's multiple-comparison post hoc analysis. Area under the curve (AUC) LH and FSH during 8 h of frequent LH sampling (every 10 min) and FSH sampling (every hour) was determined by trapezoid integration on the pre-treatment day (control) and on day 7 of NK3R antagonist administration. Comparisons of AUC across time and between the groups were performed using repeated-measures 2-way ANOVA with Bonferroni's multiple-comparison post hoc analysis. AUC LH and FSH responses to kisspeptin-10 administration were not calculated for 1 of the 11 women, as full 2-h sampling data after kisspeptin-10 injection were not obtained.

The data were tested for normality by the Shapiro-Wilk normality test. Parameters of LH pulsatility were compared by the paired Student *t* test (for normally distributed data: secretory mass per pulse, basal and pulsatile secretion, ApEn [approximate entropy, a measure of the orderliness of pulsatility]) or the Wilcoxon matched-pairs signed-rank test (for data that did not have a normal distribution: pulse frequency).

Paired mean frequencies of hot flashes and night-time awakenings were compared using the Wilcoxon matched-pairs signed-rank test. Mean flash severity scores (unpaired as the score was set to missing on the day that hot flashes equalled zero) were compared using the Mann-Whitney test. Total and individual item HFRDIS scores were compared by the Wilcoxon matched-pairs signed-rank test. The time course of hot flash frequency changes across the 7 days of NK3R antagonist treatment was analysed by the Friedman test followed by Dunn's multiple-comparison post hoc analysis. Data are presented as mean ± SEM.

Results

MLE4901 was well tolerated with no treatment discontinuations. The haematology and biochemistry safety parameters remained stable in all subjects throughout the study period. Tablet returns did not indicate any non-compliance.

NK3R Antagonism Decreases LH but Not FSH Secretion

MLE4901 had an overall suppressive effect on LH secretion when measured once daily (ANOVA $p = 0.008$), with a significant fall after 7 days of treatment (pre-treatment 29.5 ± 4.1 vs. 24.4 ± 3.8 IU/L on day 7, $p < 0.05$; Fig. 2a). There was no difference from pre-treatment on other sampling days. However, a more detailed analysis of 10-min sampling of LH secretion over 6 h pre-treatment and on day 7 of NK3R antagonist administration showed no difference in AUC LH (Fig. 2b). FSH secretion was unchanged by the NK3R antagonist (Fig. 2c).

NK3R Antagonism Modulates Pulsatile LH Secretion in Postmenopausal Women

Examples of LH pulse frequency profiles are shown in Figure 3a, g. Deconvolution analysis showed that NK3R antagonist treatment decreased basal (i.e., non-pulsatile) LH secretion ($p = 0.006$), although the LH pulse frequency did not change with the NK3R antagonist in the group as a whole (0.8 ± 0.1 vs. 0.7 ± 0.1 pulses/h, ns) (Fig. 3b, c). The secretory mass per LH pulse was increased with NK3R antagonist treatment ($p = 0.01$) (Fig. 3d), with no overall effect on the total amount of LH secreted in a pulsatile manner (Fig. 3e), and there was no change in ApEn (Fig. 3f).

However, in a secondary analysis of the 8 women with self-reported symptomatic hot flashes, NK3R antagonist treatment reduced both basal LH secretion ($p = 0.03$) and the frequency of LH pulses (from 1.0 ± 0.1 to 0.7 ± 0.1 pulses/h, $p < 0.05$) (Fig. 3h, i). The mass of LH per pulse was increased ($p = 0.04$), although pulsatile LH secretion remained unchanged (Fig. 3j, k). The orderliness (ApEn) of the LH secretion pattern was unaffected by the treatment in those with flash symptoms (Fig. 3l).

Kisspeptin-10 Does Not Elicit a Gonadotropin Response with or without the NK3R Antagonist

Intravenous kisspeptin-10 was administered at 6 h during the frequent-sampling protocol, with 2 h of sampling thereafter. Kisspeptin-10 had no effect on AUC LH or FSH secretion in postmenopausal women, both before and after 7 days of treatment with the NK3R antagonist (Fig. 4).

NK3R Antagonism Reduces Self-Reported Postmenopausal Hot Flashes

Hot flash frequency, severity, and interference with daily activities were recorded for 7 days before treatment and whilst taking the NK3R antagonist in the 8 postmenopausal women experiencing these symptoms. These women reported a reduction in hot flash frequency (from 3.4 ± 1.2 to 1.0 ± 0.6 hot flashes/day, $p = 0.008$) and night-time awakenings caused by flashes (from 1.6 ± 0.3 to 0.4 ± 0.2 awakenings/night, $p = 0.008$) during the 7 days of taking the NK3R antagonist (Fig. 5a, b). There was also an improvement in the severity of the flashing symptoms from moderate to mild (mean severity 2.1 ± 0.2/3 vs. 1.4 ± 0.1/3, $p = 0.03$) with the NK3R antagonist (Fig. 5c). The time course of changes in daytime hot flashes across the 7 days of NK3R antagonist administration was analysed ($p < 0.0001$), showing a significantly lower hot flash frequency on the 2nd day of treatment ($p < 0.05$ vs. baseline) and thereafter each day for the remainder of NK3R antagonist administration ($p < 0.05$ at days 3–7 vs. baseline) (Fig. 5d). Night-time awakenings as a result of those flashes also showed a rapid decrease in frequency with the NK3R antagonist, and were reduced throughout treatment ($p = 0.0003$) (Fig. 5e).

The NK3R antagonist reduced the total HFRDIS score (from 31.3 ± 7.7 to 9.0 ± 4.7, $p = 0.008$), indicating a significantly reduced interference of hot flashes with daily function (Table 1). The highest mean interference rating was noted for sleep, which was improved by the NK3R antagonist (from 6.4 ± 0.8 to 1.1 ± 0.6, $p = 0.008$). Whilst all other individual HFRDIS items also showed reductions in their interference scores, these reductions did not reach statistical significance.

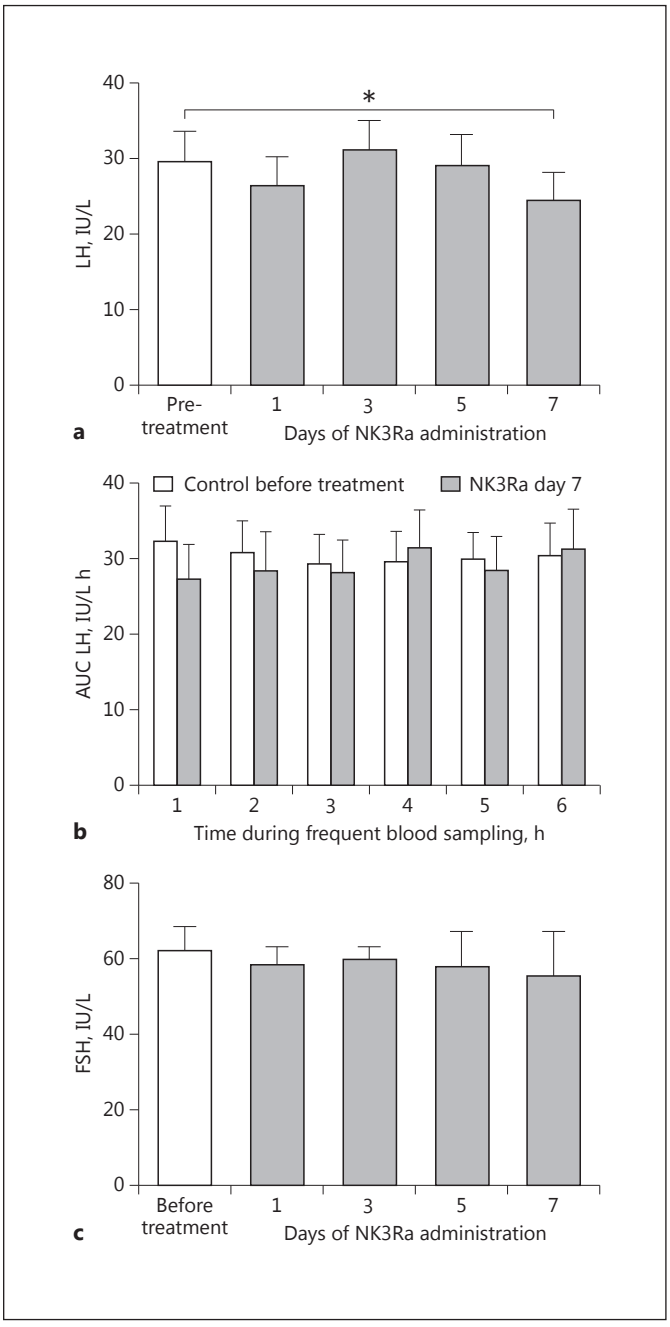

Fig. 2. Gonadotropin response to administration of the neurokinin 3 receptor antagonist (NK3Ra) in healthy postmenopausal women ($n = 11$). **a** Luteinising hormone (LH) concentrations during the 7-day course of NK3Ra treatment (blood samples taken once daily). **b** Area under the curve (AUC) LH during 6 h of 10-min LH sampling before treatment and on day 7 of NK3Ra administration. **c** Follicle-stimulating hormone (FSH) response to NK3Ra. Data are presented as mean ± SEM. * $p < 0.05$ versus pre-treatment.

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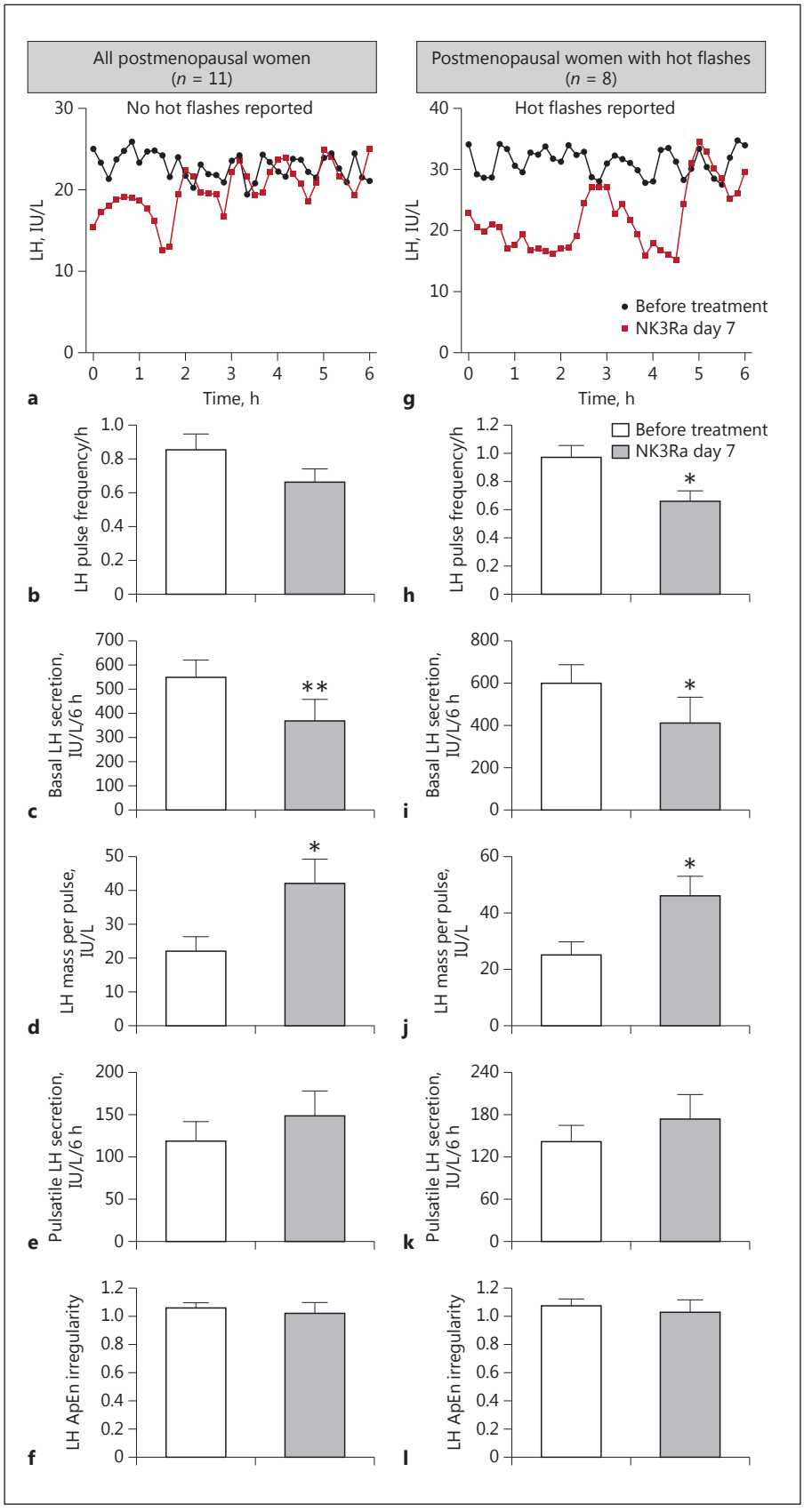

Fig. 3. Analysis of 6-h luteinising hormone (LH) secretion pattern on day 7 of neurokinin 3 receptor antagonist (NK3Ra) treatment compared to control before treatment in healthy postmenopausal women as a group (**a–f**; $n = 11$) and in only those experiencing hot flashes (**g–l**; $n = 8$). **a**, **g** Illustrative LH pulse profile from 2 subjects (**a**, with no flashes; **g**, with flashes) undergoing 10-min blood sampling for LH for 6 h with no NK3Ra treatment (black dots) and on day 7 of NK3Ra treatment (red squares). **b–f** Mean LH pulse frequency (**b**), basal (non-pulsatile) LH secretion (**c**), mass of LH per pulse (**d**), pulsatile LH secretion (**e**), and the relative orderliness/regularity (ApEn) of the LH secretion pattern (**f**) on day 7 of NK3Ra treatment were compared to before treatment in all postmenopausal women ($n = 11$). **h–l** Mean LH pulse frequency (**h**), basal (non-pulsatile) LH secretion (**i**), mass of LH per pulse (**j**), pulsatile LH secretion (**k**), and the relative orderliness/regularity of the LH secretion pattern (**l**) on day 7 of NK3Ra treatment were compared to before treatment in those postmenopausal women reporting hot flashes ($n = 8$). Data are presented as mean ± SEM. * $p < 0.05$, ** $p < 0.01$.

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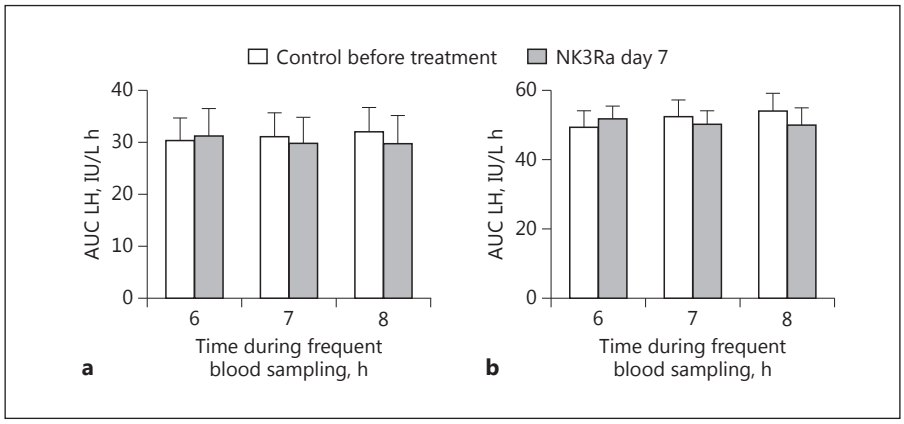

Fig. 4. Area under the curve (AUC) luteinising hormone (LH; **a**) and AUC follicle-stimulating hormone (FSH; **b**) response to kisspeptin-10 injection with and without the neurokinin 3 receptor antagonist (NK3Ra) in postmenopausal women (n = 10). AUC LH (sampling every 10 min) and AUC FSH (sampling every hour) were compared over 1 h before kisspeptin-10 administration (i.e., after 6 h) with 2 h after kisspeptin-10 administration, before NK3Ra treatment, and after 7 days of NK3Ra treatment during frequent blood sampling. Data are presented as mean ± SEM.

Table 1. Differences in HFRDIS scores before treatment and during 7 days of NK3Ra administration

Hot flash interference	Before treatment (n = 8)	NK3Ra (n = 8)	p value
Total HFRDIS score	31.3 (7.7)	9.0 (4.7)	0.008
Individual HFRDIS item responses (0–10)[1]			
Work	5.8 (1.1)	1.8 (1.1)	0.25
Social activities	5.5 (1.0)	1.5 (1.2)	0.13
Leisure activities	4.8 (0.9)	1.5 (1.2)	0.14
Sleep	6.4 (0.8)	1.1 (0.6)	0.008
Mood	4.8 (0.8)	1.4 (0.7)	0.06
Concentration	5.8 (0.8)	1.6 (0.7)	0.06
Relations with others	2.8 (1.1)	0.3 (0.3)	0.25
Sexuality	4.8 (1.0)	2.6 (1.2)	0.25
Enjoyment of life	3.7 (0.8)	1.2 (0.6)	0.06
Overall quality of life	4.2 (0.7)	1.3 (0.6)	0.06

Total and individual item HFRDIS scores in 8 postmenopausal women are shown. Data are presented as mean ± SEM. HFRDIS, Hot Flash-Related Daily Interference Scale. [1] 0 = do not interfere; 10 = completely interfere.

Discussion

This study has investigated the role of NKB and its interaction with kisspeptin in the regulation of GnRH and LH secretion in postmenopausal women. Selective blockage of NK3R signalling had a limited suppressive effect on LH secretion as assessed by once-daily sampling, with no detected effect on FSH release. Blood sampling during drug administration was done before the morning dose; as the half-life of MLE4901 (termed AZD4901 at that time) is approximately 8.5 h [26], this may have reduced the ability of this sampling schedule to detect suppression. This is supported by a detailed analysis of pulsatile LH secretion on the last day of drug administration, which revealed a marked effect of the NK3R antagonist on the parameters of pulsatile secretion (Fig. 3), which were measured over 6 h immediately following drug administration. This confirms a role for NK3R signalling in mediating the LH hypersecretion characteristic of the postmenopausal state. The physiological role of NKB in pulsatile GnRH secretion was further supported in a subgroup of women experiencing hot flashes, where a reduction in LH pulse frequency was identified. These women also reported a marked reduction in flash symptoms whilst on the NK3R antagonist, which is consistent with a link between GnRH pulsatility and vasomotor symptoms [20].

These data show that, in postmenopausal women, the NK3R antagonist decreased LH but not FSH secretion. This pattern of effect of the NK3R antagonist is consistent with data from patients with inactivating mutations in NKB or its cognate receptor who display reduced LH levels but near-normal FSH secretion, likely mediated through a decreased GnRH pulse frequency [11]. While the present study has shown that, in postmenopausal women, high LH output can be altered by the NK3R antagonist, the LH pulse frequency in the group as a whole, the proportion of total LH secreted in pulses, and the regularity of LH secretion were not affected. The mass of LH secreted per pulse was increased with the NK3R antagonist, although this may have been due to LH pulses merging in postmenopausal women without return to basal secretion, thereby giving an apparently greater LH secretory mass per pulse. These findings differ from the decreased frequencies of LH pulses seen in women with PCOS [12], in premenopausal women [8], and in ovariectomised ewes [18]. The duration and/or dose of NK3R

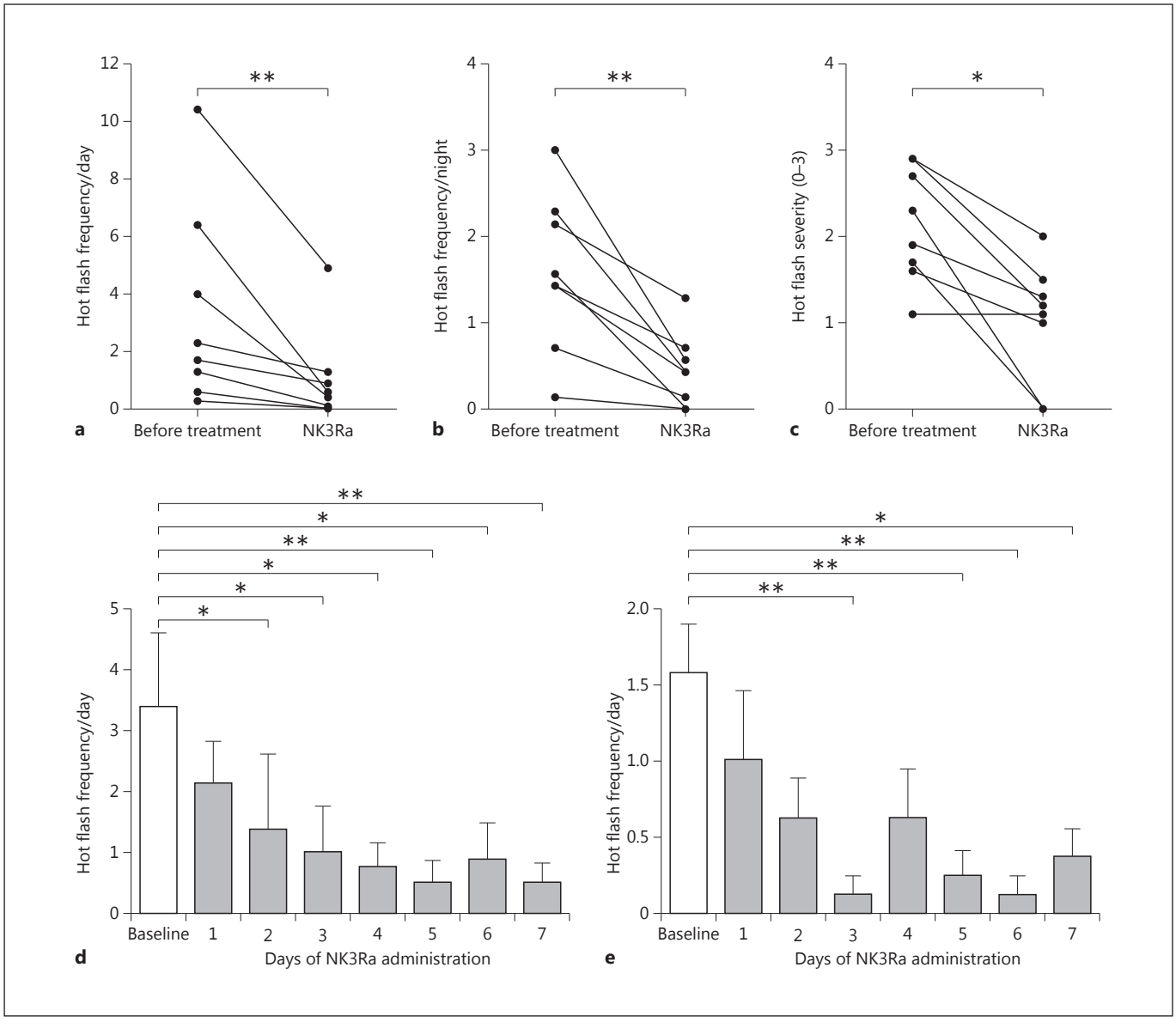

Fig. 5. Differences in the frequency and severity scores of menopausal hot flashes before treatment and during 7 days of neurokinin 3 receptor antagonist (NK3Ra) administration ($n = 8$). **a–c** The response to NK3Ra is shown for individual postmenopausal women for the frequencies of daytime (**a**) and night-time (**b**) hot flashes and their severity (**c**), showing the mean over 7 days before and during NK3Ra administration. **d**, **e** The time course of reductions in mean daytime (**d**) and night-time (**e**) hot flash frequencies over 7 days of NK3Ra administration is compared to the mean of the pre-treatment period. Data are presented as mean ± SEM. * $p < 0.05$, ** $p < 0.01$, versus baseline.

antagonist administration in this study may have contributed to the limited suppressive effect seen on LH/GnRH secretion in contrast to hypogonadotropic hypogonadism observed in patients with loss-of-function mutations in NKB and its receptor [11] or NK3R antagonist administration for 4 weeks to women with PCOS [12].

The more limited effect of the NK3R antagonist on the high LH secretion in menopausal women may also reflect the consequences of long-term oestrogen depletion, since increased *TAC3* expression has been reported in the hypothalamus of postmenopausal women, with hypertrophy of NKB neurones. Increased expression of hypothalamic *Tac3* mRNA after ovariectomy in animal models is

restored with oestrogen replacement [27–29], and sex steroid receptors have been localised to NKB perikarya [14, 15]. These data clearly support a role for oestrogenic feedback in the regulation of NKB signalling in the hypothalamus, and it seems plausible that loss of negative oestrogen feedback following the menopause and a subsequent increase in NKB signalling impedes the ability to manipulate the NKB/kisspeptin/GnRH pathway as readily as in other states. The duration of oestrogen withdrawal as well as differences between species complicate the direct comparison of these results, especially since there is anatomical variation in pathways mediating sex steroid feedback between humans and other mammals [13, 30–32].

Interestingly, the NK3R antagonist reduced the LH pulse frequency in postmenopausal women reporting hot flashes. It is, however, unclear whether NKB acts to modulate LH/GnRH pulsatility specifically in those experiencing flash symptoms, as larger groups would be required to explore this more robustly. In this study, postmenopausal women were not recruited based on having hot flashes, and statistical comparisons between those with and those without flashing were not feasible, since there were only 3 women in the latter subgroup. The small sample size may have impacted overall effects seen on LH pulsatility. It is, however, possible that the NKB/GnRH pathway is somewhat different and is enhanced in women with hot flashes, thereby being more responsive to suppression by the NK3R antagonist. NKB signalling may therefore be a common pathway regulating both vasomotor symptoms and GnRH/LH pulsatility.

The lack of effect of kisspeptin-10 on gonadotropin secretion seen in this study is consistent with previous data showing a minimal LH response to both kisspeptin-10 and GnRH injection in postmenopausal women as opposed to women in the early follicular phase of the menstrual cycle or those taking hormonal contraceptives [6], or during oestrogen administration, where this dose of kisspeptin-10 was able to induce surge-like LH secretion [8]. In premenopausal women, the response to kisspeptin-10 is directly and positively related to serum oestradiol concentrations [7, 8]. This further highlights the role of sex steroid feedback in the control of hypothalamic neuropeptide signalling. It seems likely that due to loss of negative oestrogen feedback after menopause, the kisspeptin system is already operating at its maximum to increase GnRH and LH secretion, with little scope for any additional stimulatory effect of exogenous kisspeptin. This is supported by hybridisation histochemistry showing that in the postmenopausal infundibular nucleus kisspeptin neurones are hypertrophied, that there are more of them, and that they have increased expression of *KISS1* as well as *TAC3* mRNA [13–15]. The absence of an effect of kisspeptin-10 on LH alone or in the presence of NK3R antagonism precludes any clear analysis of the hierarchical relationship between kisspeptin and NKB in postmenopausal women, although it would be of interest to repeat the experiment in postmenopausal women taking oestrogen replacement. The same batch of kisspeptin-10 was effective in contemporaneous studies on premenopausal women [8]. In patients with inactivating mutations in *TAC3* or *TAC3R*, kisspeptin-10 was able to restore the LH pulse frequency to normal and increase LH AUC [11], and we have recently shown that in premenopausal women in a model of LH surge this NK3R antagonist truncated the response to kisspeptin-10 [8]. Thus, while NKB signalling appears to be predominantly proximal to kisspeptin signalling, as has also been indicated in animal and human studies [11, 33, 34], there is a complex interaction between these pathways.

The finding that all postmenopausal women with symptomatic flashes (n = 8) reported a reduction in the frequencies of total and night-time hot flashes and an improvement in their severity whilst on the NK3R antagonist is striking and novel. The response to the NK3R antagonist by a reduction of flash symptoms was also a rapid one, with significant falls in both daytime and nighttime symptoms after only 2 days of treatment. The NK3R antagonist also reduced the interference of those flashes with daily activities. The present data provide evidence to implicate NKB as a key link between sex steroid deficiency and hot flashes, as proposed by studies on animal models [20]. In premenopausal women, NKB itself administered as an intravenous infusion over 30 min induced the sensation of heat, which was accompanied by an increased heart rate and skin conductance, resembling events associated with menopausal hot flashes [23]. Moreover, a recent genome-wide association study has localised single nucleotide polymorphisms associated with vasomotor symptoms to the NK3R locus (*TAC3R*) [35]. Reduced cutaneous vasodilation in rodents with ablation of NKB-expressing neurones [36] and a lowered body core temperature in NK3R antagonist-treated sheep [19] lend further support to the involvement of NKB signalling in hot flashes. The reduction in frequency of LH pulses with the NK3R antagonist observed here in the group of women with flash symptoms suggests the mechanism of flashes to be tied to the hypothalamic control of pulsatile GnRH secretion. This is consistent with studies showing a synchronisation of LH pulses with hot flashes in women [20–

22]. Although these data support the involvement of NKB in vasomotor symptoms, it is possible that separate pathways exist, those that mediate hot flashes and those that are associated with pulsatile GnRH/LH secretion, with both involving NKB regulation.

The limitation of the absence of a placebo group in the present study is acknowledged, as well as the small number of women studied. Placebos have been shown to reduce hot flashes by 20–30% within 4 weeks of treatment, and in 15% of women experiencing symptomatic flashes [37, 38]. In contrast, NK3R antagonist administration in this study was associated with an approximately 70% reduction in hot flashes over only 7 days of treatment, and this in all 8 women reporting hot flashes. An objective assessment of the menopausal hot flashes, such as using skin conductance monitors, might have reinforced these findings. This group had a lower hot flash frequency than is generally found in studies specifically investigating therapies for that condition [24], reflecting recruitment criteria not specifying the presence of flashes or a minimum flash frequency.

In summary, the secretion of LH was reduced in postmenopausal women by administration of an NK3R antagonist, but the absence of a response to kisspeptin-10 precluded an investigation of the relationship between the NKB and kisspeptin pathways in postmenopause. We have shown that NK3R antagonist treatment reduced subject-reported menopausal hot flashes and reduced the frequency of LH pulses in those women reporting such flashes. These data demonstrate that NKB signalling is involved in the regulation of LH secretion in the hypergonadotropic state of the menopause and provide indirect evidence linking vasomotor symptoms and high GnRH pulse frequencies to the NKB pathway. Our study on a relatively small number of postmenopausal women suggests that NK3R antagonism may have a clinical application in the management of hot flashes.

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Disclosure Statement

J.T.G. has undertaken consultancy work for AstraZeneca and Takeda Pharmaceuticals and is an employee of Boehringer Ingelheim. R.A.A. has undertaken consultancy work for AstraZeneca and Takeda Pharmaceuticals. R.P.M. consults for Ogeda and is CEO of Peptocrine. J.D.V. and K.S. have nothing to disclose.

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