Endocrine (2019) 63:193–203
https://doi.org/10.1007/s12020-018-1777-z

MINI REVIEW

Is calcitonin gene-related peptide a modulator of menopausal vasomotor symptoms?

Maria Alice Oliveira[1] · William Gustavo Lima[1] · Dante Alighieri Schettini[1] · Cristiane Queixa Tilelli[1] · Valéria Ernestânia Chaves[1]

Received: 25 May 2018 / Accepted: 29 September 2018 / Published online: 10 October 2018

Abstract

Purpose Calcitonin gene-related peptide (CGRP) is a neuropeptide widely distributed in the central and peripheral nervous systems, which is known as a potent vasodilator. Postmenopausal women who experience hot flushes have high levels of plasma CGRP, suggesting its involvement in menopausal vasomotor symptoms.

Methods In this review, we describe the biochemical aspects of CGRP and its effects associated with deficiencies of sexual hormones on skin temperature, vasodilatation, and sweating as well as the possible peripheral and central mechanisms involved in these events.

Results Several studies have shown that the effects of CGRP on increasing skin temperature and inducing vasodilatation are potentiated by a deficiency of sex hormones, a common condition of postmenopausal women. Additionally, the medial preoptic area of the hypothalamus, involved in thermoregulation, contains over 25-fold more CGRP-immunoreactive cells in female rodents compared with male rodents, reinforcing the role of female sex hormones on the action of CGRP. Some studies suggest that ovarian hormone deficiency decreases circulating endogenous CGRP, inducing an upregulation of CGRP receptors. Consequently, the high CGRP receptor density, especially in blood vessels, amplifies the stimulatory effects of this neuropeptide to raise skin temperature in postmenopausal women during hot flushes.

Conclusions The duration of the perception of each hot flush in a woman is brief, while local reddening after intradermal administration of α-CGRP persists for 1 to 6 h. This contrast remains unclear.

Keywords Calcitonin gene-related peptide · Hot flush · Thermoregulation · Menopause

Abbreviations

AM	adrenomedullin;
AMY	amylin;
CGRP	calcitonin gene-related peptide;
CLR	calcitonin receptor-like receptor;
CREB	cAMP response element-binding;
CTR	Calcitonin receptor;
GnRH	gonadotropin-releasing hormone;
MeSH	medical subject heading;
POA	preoptic area;
PVN	hypothalamic paraventricular nucleus;
RAMP	receptor activity-modifying proteins;

✉ Valéria Ernestânia Chaves
valeria.chaves@gmail.com

[1] Laboratory of Physiology, Federal University of São João del-Rei, Divinópolis, Minas Gerais, Brazil

Introduction

Calcitonin gene-related peptide (CGRP) is a neuropeptide involved in several physiological functions, including nociception, inflammation, vasodilatation, and energy metabolism [1–4], with receptor sites both in the nervous system and other tissues [2]. CGRP is a potential modulator of menopausal vasomotor symptoms. Herein, we describe aspects of the biochemistry of CGRP and its effects associated with deficiencies of sexual hormones on skin temperature, vasodilatation, and sweating as well as the possible mechanisms involved in these peripheral events. Previously, the relation of higher levels of CGRP with the development of hot flushes in postmenopausal women has been reviewed, but little has been discussed about the role of this neuropeptide in the pathophysiology of this event [5–7]. Thus, this review is focused on the peripheral and central mechanisms by which CGRP seems to act to induce hot flushes in postmenopausal women (Table 1, Fig. 1).

Table 1 Summary of main biological effects of calcitonin gene-related peptide (CGRP) associated with menopausal symptoms

Menopausal Symptoms	CGRP effects	Model	References
Excessive sweating/night sweating [63, 64]	Increased acetylcholine-induced sweating	Humans	[40, 49]
Hot flushes [63]	Increase in skin temperature	Rats	[79, 82, 94–98]
	Change in the firing rate in slices of preoptic area and anterior regions of the hypothalamus from rats	In vitro	[122]
Flush in digits, face, arms, chest, abdomen, back, and legs [64, 69]	Induced vasodilatation	Humans, rabbits, rats	[2, 45, 49, 100–108, 110, 114]
		Norepinephrine- or prostaglandin -induced contraction (in vitro)	[45, 79, 82, 97, 111]
Palpitations [69]	Increased heart frequency	Humans	[38]

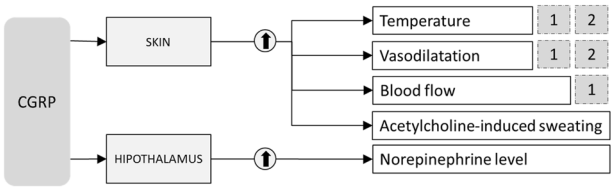

Fig. 1 Summary of the possible calcitonin gene-related peptide (CGRP) effects on flushing. CGRP is increased at the onset of hot flushes in postmenopausal women [71–73] and in men with flushes who have been castrated due to prostate cancer [76]. The upward arrow indicates stimulatory action. (1) Opposing effect induced by CGRP deficiency and/or antagonism. (2) Potentiated effect due to ovariectomy, orchiectomy, or chemical castration

Methodology

The references for the main theme of this narrative review were searched in Pubmed/Medline, Scopus, and Web of Science databases (up to 4 August 2018) using the following Medical Subject Heading (MeSH) terms: ("calcitonin gene-related peptide" OR "CGRP") AND ("hot flashes" OR "hot flushes" OR "menopause vasomotor symptoms" OR "menopause" OR "postmenopausal women"). Studies that assessed the role of CGRP in menopausal vasomotor symptoms in humans and animals were included in this review. Two reviewers conducted independent searches and included all relevant articles published in English, Spanish, French, Italian, or Portuguese, with no restrictions of publication date. Additionally, all references were screened to identify potential eligible studies that would enrich the review. Whenever necessary, a new search was conducted, mainly in Pubmed/Medline, to find articles investigating biochemical, pharmacological, and/or physiological aspects of CGRP and/or its antagonists, as well as sexual hormone effects on synthesis, release, or functions of CGRP.

Biochemical and pharmacological aspects of CGRP and its receptors

Described for the first time in 1982, CGRP is a 37-amino acid neuropeptide that belongs to the calcitonin peptides family which also includes amylin (AMY), adrenomedullin (AM), adrenomedullin 2 (AM2, also known as intermedin), and calcitonin [8]. In humans, CGRP is encountered as two isoforms known as α- and β-CGRP which differ in only three amino acids [9]. Both isoforms are encoded by chromosome 11, but in distinct gene regions [10]. α-CGRP is produced by tissue-specific alternative splicing of the primary transcript of the *CALCA* gene located in the q14-pter region [11], while β-CGRP is encoded by a distinct *CALCB* gene located in the q12-pter portion [10]. CGRP is one of the most broadly distributed peptides in nervous tissues, being expressed in motor areas, somatic afferents,

cerebellum, basal ganglia, thalamus, hypothalamus, and neurons in the ventral horn of the spinal cord [12]. Additionally, CGRP immunoreactivity is found in peripheral organs, such as heart, thyroid, lung, gastrointestinal tract, and blood vessels, extending from the adventitia to the medial muscle layers [2]. Plasma CGRP is considered a result of an "overspill" from perivascular sensory neurons, and the major effects of CGRP are exerted locally in the vessel wall close to its release site [2].

CGRP receptors are common class B G protein-coupled receptors that interact with receptor activity-modifying proteins (RAMPs) to allow its adequate function and pharmacology [13]. Calcitonin receptor (CTR) or calcitonin receptor-like receptor (CLR, previously known as CRLR) is associated with one of three RAMPs (RAMP1, 2, and 3) to form several heterocomplexes that show differential affinity to each peptide of the calcitonin family [14]. The CLR/RAMP1 complex has high affinity to CGRP and is called the CGRP receptor [15]. CTR/RAMP1, CTR/RAMP2, and CTR/RAMP3 complexes are, respectively, AMY_1, AMY_2, and AMY_3 receptors [15]. It has been demonstrated that CGRP binds to the AMY_1 receptor with close to equal activity at CGRP receptors (CLR/RAMP1) [15]. CLR/RAMP2 and CLR/RAMP3 are AM receptors (AM_1 and AM_2) and have weak affinity for CGRP [15]. The CGRP receptor (CLR/RAMP1) is highly sensitive to its antagonist $CGRP_{8–37}$ [16]; however, this compound also inhibits AM and AMY responses, in lower degrees [15]. More recently, monoclonal antibodies neutralizing CGRP or blocking its receptors and small molecule CGRP antagonists were developed. In 2018, the U.S. Food and Drug Administration approved Erenumab (AIMOVIG™, erenumab-aooe), which is the first antagonist of CGRP licensed for clinical use [17]. This subcutaneous formulation, developed by Amgen and Novartis, is a fully human monoclonal to antibody CGRP receptor for the prevention of migraine in adults [18]. Currently, a double-blind, placebo-controlled trial (phase I) is being conducted with Erenumab to evaluate the prevention of hot flushes in menopausal women aged 45–65 years, thus highlighting the therapeutic potential of CGRP antagonism in vasomotor symptoms associated with mild-age women [19]. In addition, oral formulations containing CGRP receptor antagonist have been developed and evaluated in clinical trials for different migraine types. From 2004, when olcegepant, the first oral antagonist of CGRP receptor, was efficiently tested against acute treatment of migraine, five other agents (i.e., telcagepant, MK-3207, BI44370, ubrogepant, and rimegepant) have been tested [20]. These compounds, known as gepants, produce significantly better results when compared with placebo in the treatment of several types of headache in clinical and preclinical studies, including migraine associated with the female sexual cycle. To evaluate the safety, tolerability, and efficacy of telcagepant (i.e., MK-0974) in the prevention of menstrual cycle-related migraine, a 6-month phase II/III, randomized, double-blind, placebo-controlled clinical trial was developed [19]. However, although telcagepant and other CGRP receptor antagonists, such as MK-3207 and BI44370, are effective and usually well tolerated, clinical studies show that these drugs induce adverse events; thus, the development of these formulations as an acute or prophylactic treatment for migraine has been discontinued [21–23].

CGRP receptor binding sites are found in many of the sites where immunoreactive axons terminate [24]. However, the distribution pattern of CGRP receptors does not strictly follow the label of axons immunoreactive to CGRP in the nervous tissue [24]. For example, the main sensory systems reveal a variety of CGRP receptor binding site patterns, only a few of which can be related to sites of CGRP-immunoreactive axon terminals [24]. There is moderate labeling of ^{125}I-CGRP in the visual and auditory area of rats, becoming more intense in the gustatory area [25]. Additionally, in the thalamus and hypothalamus, only low densities of CGRP receptors have been reported [12]. CGRP receptors are also found in several limbic system areas, such as the amygdala, cingulate cortex, and ventral striatum [24]. Although CGRP action has been interpreted in terms other than conventional synaptic effects [24], we must emphasize that the use of ^{125}I-CGRP allows non-specific labeling due to cross-reactivity of CGRP with AMY_1 and AM receptors [15] and that abluminal administration of CGRP induces vasodilation, but not luminal [26], suggesting that CGRP do not cross the blood–brain barrier. In addition, AM activates all three CLR/RAMP complexes, including the CGRP receptor [27].

Several peripheral tissues [28–34] also show labeling for CGRP receptors, inducing several biological functions [35–44]. Among these functions, this neuropeptide induces vasodilation [2, 45–47] and sweating [48, 49] as well as modulation of autonomic tonus [50], events present in menopausal vasomotor symptoms (Table 1, Fig. 1).

Menopause and hot flushes

Menopause is characterized by loss of ovarian follicle development, clinically defined as a year of amenorrhea that is not related to any surgical or drug treatment [51–53]. Natural menopause arrives mainly in women around their mid-50s [54], but premature menopause might involve younger women, being an important biomarker for chronic disease and mortality [55, 56]. In fact, earlier menopause is associated with an increased risk of cardiovascular disease [57], obesity [58], and osteoporosis [59], but it is also an important protector factor for breast,

endometrial, and ovarian cancers [60]. There are 470 million women older than the average age for natural menopause, and it is estimated that 1.2 billion women will be menopausal or postmenopausal worldwide by the year 2030 [51, 61]. Although some of these women may be asymptomatic, more than 85% show postmenopausal-associated disorders [52].

In the menopausal transition, serum levels of estradiol and progesterone decrease and follicle-stimulating hormone levels increase, resulting in physiological changes and clinical symptoms [62]. The most prevalent symptoms among peri- and postmenopausal women are hot flushes and night sweats, known as vasomotor menopausal symptoms [63]. Hot flushes are typically described as sudden sensations of warmth and flush, beginning in the upper thorax and neck, rising into the face, and extending down the arms [64]. The occurrence of typical hot flush episodes during sleep is termed night sweats [64]. Some authors suggest that these night events lead to complaints such as irritability, anxiety, nervousness, depression, fatigue, forgetfulness, and inability to concentrate [64]. Contrary to this viewpoint, others suggest direct effects of hormonal level changes and brain function decay in postmenopausal individuals, both in animal and human models [65, 66]. Despite being extensively researched, the pathophysiology of hot flushes is still poorly understood [67, 68]. The first systematic investigation on body temperature changes during menopausal hot flushes describes that internal temperatures decrease after each flush [69]. In the skin area where sweating occurs, the temperature decreases during and increases after the flush [69]. Finger and toe temperatures always show a sharp increase at the onset of a flush, followed by a slower decrease [69]. Only the cheeks show additional temperature increases [69]. Additionally, the heart frequency increases at the onset of the flush, but decreases immediately thereafter [69].

Sex hormones influence on CGRP levels

Plasma CGRP levels are higher in healthy women than in men [70] and increase during the onset of a hot flush in postmenopausal women [71–73] (Fig. 1). Among women who experience hot flushes, postmenopausal women have significantly higher CGRP levels than premenopausal women [74]. In addition, 24 h urinary excretion of CGRP is higher in women with vasomotor symptoms compared to non-flushing women [75]. Increased plasma CGRP is also documented in men with flushes who have been castrated due to prostate cancer [76] (Fig. 1). These findings suggest that sexual hormones regulate CGRP levels and that this neuropeptide may be involved in hot flushes. However, plasma and 24 h urinary CGRP levels are not affected by the menstrual cycle phases [74, 75], although body temperature is higher in the luteal phase compared to the follicular phase. There is also no difference in CGRP levels among pre- and postmenopausal women who were not having hot flushes [74] and among healthy aging men during flushes [77].

The evidence for sex hormones influence on CGRP levels is more extensively shown in rodents. Plasma CGRP is lower in middle-aged female and ovariectomized rats, when compared to young controls or sham-operated rats [78, 79]. In addition, in experiments of hormone replacement therapy, where ovariectomized rats receive estradiol or progesterone treatments, plasma CGRP levels increase [70, 78, 80–82]. Chronic perfusion of sex steroid hormones to ovariectomized rats also increase CGRP-immunoreactive cells [78]. Furthermore, CGRP messenger RNA (mRNA) synthesis increases in sensory neurons of ovariectomized rats treated with estrogen in a dose-related manner, an effect blocked by an estrogen receptor antagonist [83]. High-dose estrogen treatment or estrogen supplement after ovariectomy induces a large increase of CGRP immunoreactivity and CGRP-immunoreactive cell to LH cell ratio in the anterior pituitary [84]. In this gland, CGRP immunoreactivity is localized mainly in gonadotrophs, and α-CGRP and β-CGRP mRNAs are detected in CGRP-immunoreactive cells [84].

Additionally, in vitro studies also show that sex hormones modulate CGRP release and mRNA expression. The release of CGRP is increased in the murine medullary thyroid carcinoma C-cell line when treated with estradiol alone [85]. Moreover, it was also demonstrated that 17 β-estradiol induces CGRP release from F11 cells, a somatic cell hybrid of a rat embryonic dorsal root ganglion and mouse neuroblastoma cell line N18TG2 [86]. Conversely, ovariectomized rats present with a marked increase (600%) in CGRP mRNA expression in trigeminal ganglia tissue compared to control rats [87]. This induced mRNA level is lowered down to 150% following the administration of estrogen to ovariectomized rats [87].

Ovariectomy decreases the expression of phosphorylated cAMP response element-binding (CREB) protein in hippocampus of rats, while the replacement with 17β-estradiol throughout the post-ovariectomy period prevents this effect [88]. The treatment with 17 β-estradiol 15 days after ovariectomy for 7 days or 21 days also reverts the effect of ovariectomy [88]. It was previously demonstrated that intracerebroventricular administration of estradiol increases phosphorylated CREB in rat hippocampal CA1 region [89]. Similar results are found in human prostate and breast cancer cells and in rat dorsal root ganglion [90–92]. These findings suggest that CREB can be involved in the estradiol effects on CGRP mRNA expression, because the binding of CREB to the cAMP response element is necessary and

sufficient for the activation of the CGRP promoter by cAMP [93].

Peripheral effects of CGRP

Skin temperature

Mice lacking αCGRP ($CGRP^{-/-}$) at 49 days of age have a lower surface temperature compared with $CGRP^{+/+}$ mice [94] (Fig. 1). In addition, peripheral α-CGRP (10 μg kg^{-1} intravenous (i.v.)) administration increases the skin temperature in rats (by as much as 20%) at 40 to 50 min after injection [79], a dose-dependent effect [95] (Fig. 1). The same dose also induces skin temperature increases in anesthetized female rats, reaching maximal values at 20 to 60 min after injection [96]. After increasing, the temperature gradually declines, in particular the temperature of the ear that falls below the basal level [96]. Interestingly, skin temperature elevation induced by CGRP is significantly higher in ovariectomized rats than in sham-operated rats [79, 82, 95, 97] (Fig. 1). The CGRP-induced increase in skin temperature in rats is inhibited by pretreatment with its antagonist $CGRP_{8–37}$ (100–1000 μg kg^{-1} intraperitoneal) in a dose-dependent manner [79, 96] (Fig. 1). Treatment with a gonadotropin-releasing hormone (GnRH) analog, a chemical form of castration, increases CGRP-induced elevation of skin temperature [98] (Fig. 1). Subcutaneously (s.c.) injected 17 β-estradiol (0.01 mg kg^{-1}) blocks the CGRP effect on skin temperature in ovariectomized rats [82, 95] and in GnRH analog-treated rats [98]. Similar results are found in male rats: the CGRP-induced elevation of skin temperature is significantly greater in the castrated rats, both by bilateral orchiectomy or by single injection of a GnRH analog, than in sham-treated rats [99] (Fig. 1). This potentiation is inhibited by treatment with ovarian hormones as well as by testosterone replacement [99]. Conversely, intracerebroventricular injection of CGRP (10 μg kg^{-1}) does not change the tail skin temperature [96], suggesting that those effects are due to the peripheral, not central, action of this neuropeptide.

Vasodilation

The fibers that innervate blood vessels in mammalian skin release CGRP, substance P, and several other peptides [100]. Primary afferent neurons are responsible for this peptidergic innervation, thus the retrograde labeling of dorsal root ganglion cells, as well as the CGRP and substance P levels in skin, is considerably reduced after sensory chemical denervation by capsaicin [100]. Among the several biological roles in the microvascular system of the skin, CGRP stands out for its potent vasodilatory action [2, 45, 101]. Pioneer works have shown that intradermal injection of CGRP (10^{-12} mol) induces vasodilatation and increases blood flow (Fig. 1) in rabbits and humans [45, 102], where a persistent and intense hyperemia response is induced, lasting for 1 to 6 h, depending on the administered dose (15 fmol to 15 pmol range) [45]. CGRP (10^{-12} mol) relaxes the norepinephrine-induced arteriolar contraction [45], as well as the prostaglandin F2α-induced contraction of mesenteric vascular beds isolated from rats (Fig. 1) in a dose-dependent (10^{-11}–10^{-9} mol) manner [79, 97]. $CGRP_{8–37}$ (400 nmol kg^{-1}, i.v.) inhibits the increased blood flow induced by intradermal CGRP (10 pmol) in rat abdominal skin [103] (Fig. 1), induced by saphenous nerve stimulation in rat hind paw [103] and induced by trigeminal ganglion stimulation in rat facial skin [104]. More recently, it was demonstrated that telcagepant and MK-3207, as well as erenumab, inhibit the capsaicin-induced dermal blood flow in healthy and/or migraine human subjects or rhesus monkeys [105–108] (Fig. 1). This capsaicin-induced vasodilation in the human forearm skin is largely mediated by CGRP, but not by vasodilating prostaglandins, nitric oxide, or substance P [109].

As also seen in its skin temperature effect, the CGRP-induced relaxation of mesenteric vascular beds isolated from rats is significantly greater in tissue extracted from ovariectomized rats than in those from sham-operated rats [79, 97] (Fig. 1) and is blocked by 17 β-estradiol (0.01 mg kg^{-1} s.c.) [82]. Similarly, periarterial nerve stimulation or CGRP induce greater vasorelaxation in isolated tail arteries of ovariectomized rats when compared to those of sham-operated rats [110] (Fig. 1). Previously, it was demonstrated that human $CGRP_{8–37}$ also inhibits relaxation in these vessels [111] (Fig. 1). Moreover, the maximal number of ^{125}I-CGRP binding sites in isolated mesenteric arteries is approximately 3.5 times higher in ovariectomized rats than in sham-operated rats [79]. This is accompanied by a decrease in plasma CGRP levels after ovariectomy [79]. These findings suggest that the decrease in circulating endogenous CGRP after ovarian hormone deficiency may induce upregulation of CGRP receptors, consequently amplifying the stimulatory effects of CGRP to raise skin temperature [79].

$CGRP^{-/-}$ mice present an increase in peripheral vascular resistance (Fig. 1), accompanied by an increase in arterial pressure and heart rate [112, 113]. Conversely, while in humans, peripheral CGRP (25.3 nmol i.v.) administration decreases arterial pressure, an increase in the heart rate and severe facial flushing are observed [38]. In addition to the face, systemic intravenous infusion of CGRP (104–520 pmol min^{-1}) also induces a dose-dependent flush in the neck, upper trunk, and upper arms [114]. These effects are accompanied by an increase in plasma norepinephrine and epinephrine levels [38]. Intradermal

administration of 0.016 µg human α-CGRP induces vasodilatation after 1 min (Fig. 1), manifesting as local reddening; this effect persists for 5 to 6 h [45]. Infusion of CGRP (11–216 pmol min^{-1}) into the brachial artery in men induces a dose-dependent increase in the forearm blood flow and cutaneous blood flow, persisting for up to 90 min after the end of infusion [114] (Fig. 1). It has been suggested that CGRP increases regional blood flow to the brain and skin at the expense of the gastrointestinal tract [115]. Finally, CGRP also increases the acetylcholine-induced skin blood flow in humans [49] (Fig. 1).

Sweating

In humans, CGRP (10^{-7}–10^{-9} M) increases acetylcholine-induced sweating (Fig. 1) and decreases nicotine-induced sweating, while CGRP alone is insufficient to induce sweating [40, 48, 49]. CGRP-like immunoreactivity is present at the terminals of the cholinergic nerve around human sweat glands, which corroborates the hypothesis that CGRP increases cholinergic-induced transpiration, as a consequence of the vasodilation caused by CGRP [48, 116]. Thus, the influence of CGRP on acetylcholine function may, at least in part, explain the sweating that accompanies hot flushes.

Central effects of CGRP

The primary center for neural control of thermoregulation in mammals is the preoptic area (POA) of the anterior hypothalamus [117] which integrates central and peripheral signals in order to activate reflex autonomic and somatic responses for core temperature adjustments [118]. It has been suggested that postmenopausal women with hot flushes present with a reduced thermoneutral zone, triggering autonomic responses for reducing body temperature after small elevations in core temperature [119].

The medial POA of female rodents contains over 25-fold more CGRP-immunoreactive cells compared with the medial POA of males [120]. Gonadectomy of male rats increases the number of CGRP-immunoreactive cells in the medial POA, an effect decreased by administration of testosterone [120, 121]. Ovariectomy 1 month earlier, with or without subsequent 17 β-estradiol treatment, has no effect on the number or distribution of CGRP-immunoreactive cells in the medial POA [120]. However, testosterone treatment in female rats decreases CGRP cell numbers in this area [121]. These findings suggest a central role for CGRP in regulating body temperature and show that testosterone regulates CGRP in the medial POA.

CGRP (10 µM) changes the firing rate in slices of POA and anterior regions of the hypothalamus from rats [122]. Either an increase in the firing rates of temperature-insensitive neurons or a decrease in the firing rates of warm-sensitive neurons induce a shift in the thermoregulatory set point to a higher temperature [122]. Conversely, CGRP administration (1.6 pmol; 0.2 µl) into the POA did not increase the colonic and interscapular brown adipose tissue temperature in urethane-anesthetized rats [123]. However, α-CGRP (0.05 and 0.25 nmol) administration into the hypothalamic paraventricular nucleus (PVN) increases the plasma level of norepinephrine (Fig. 1), but not that of epinephrine, in a dose-dependent manner in male Wistar rats [50], suggesting CGRP regulation of sympathetic outflow that does not rely on adrenal activity. Indeed, this effect is not affected by bilateral adrenalectomy [50]. A similar increase in the plasma level of norepinephrine, as well as a significant increase in arterial blood pressure, is observed after α-CGRP (0.05 nmol) administration into the POA, anterior hypothalamus, dorsomedial hypothalamus, and ventromedial hypothalamus [50] (Fig. 1). However, the most prominent increase was caused by its microinjection into the PVN [50]. However, $CGRP^{-/-}$ mice present an increase in sympathetic nervous activity [112]. Increased central norepinephrine may be involved in human hot flushes, as they are triggered by increased brain noradrenergic release in symptomatic menopausal women who received yohimbine, an α2-adrenergic antagonist [124]. In contrast, it has also been suggested by others that a reduction in norepinephrine reuptake, using the serotonin-norepinephrine reuptake inhibitor desvenlafaxine, ameliorates hot flushes and other menopausal symptoms, instead of triggering them [125].

In response to increased body temperature in humans, the reflex mechanism involved in the heat dissipation is activated [117]. Sympathetic cholinergic nerves, which are also activated during increases in core temperature, are involved in sweating, although not always with the same time course as active vasodilation in the skin [117]. There is limited available detail on the central nervous system pathways regulating human sweating, as animal models are usually furry mammals that do not depend on sweat for heat control [126], but it is known that both hypothalamic thermoregulatory sympathetic pathways and the limbic system (via modulatory fibers) can participate in the stimulation of sweat glands [127]. CGRP receptors and estrogen receptors are present in the same brain areas, such as the hypothalamus, amygdala, cingulate cortex, and ventral striatum [24, 65, 128, 129]. Interestingly, studies using magnetic resonance images, comparing symptomatic and asymptomatic amenorrheic women, show that hot flushes are associated not with POA but with medullar as well as insular, cingulate, and frontal cortices activation [130, 131].

Concluding remarks

The elevation of skin temperature induced by CGRP during hot flushes may be mediated by the increased blood flow following the vasodilatory action of this peptide in the skin [79]. Thus, we believe that the use of monoclonal antibodies blocking CGRP receptors and/or small molecule CGRP antagonists can prevent the menopausal vasomotor symptoms, considering that these antagonists inhibit the capsaicin-induced dermal blood flow [105–108]. However, although decreases in circulating endogenous CGRP after ovarian hormone deficiency may induce upregulation of CGRP receptors [79], there is no difference in CGRP levels among pre- and postmenopausal women who were not having hot flushes [74]. In addition, CGRP levels are increased among women who experience hot flushes [74]. Thus, new experiments are needed to firmly establish this hypothesis. Several studies show that CGRP increases during the onset of a hot flush [71–73]; however, the duration of the perception of each hot flush is brief [64], while local reddening after intradermal α-CGRP administration persists for 1 to 6 h [45]. Peripheral CGRP administration induces severe facial flushing accompanied by a decrease in the arterial pressure and an increase the heart rate [38], suggesting a reflex activation of the sympathetic nervous system. Central CGRP administration induces sympathetic activation independently of the adrenal medulla, accompanied by an increase in arterial pressure [50]. At the onset of the flush, the heart rate increases and immediately thereafter decreases [69], but without changes in the blood pressure [64]. Recently, a meta-analysis showed that women with hot flushes, compared to those without, tend to have significantly higher levels of blood pressure, albeit non-significant [132]. In addition, the presence of CGRP in limbic system areas [24, 133–136] suggests its participation in the expression of behaviors associated with symptoms of postmenopausal women presenting hot flushes, such as anxiety, sleep disturbs, depression, and irritability. Thus, there is evidence that CGRP may be a key component in the pathophysiology of hot flushes (Table 1, Fig. 1), but these are complex phenomena that remain to be completely understood, mainly in humans.

Acknowledgements The authors would like to thank Renato Helios Migliorini (in memoriam) for being an exemplary scientist and professor.

Funding This work was supported by the Federal University of São João del-Rei. W.G.L. received a fellowship from the Fundação de Amparo à Pesquisa do Estado de Minas Gerais (FAPEMIG).

Author contributions All authors contributed to the development, analysis, and drafting of this article.

Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

References

1. S. Iyengar, M.H. Ossipov, K.W. Johnson, The role of calcitonin gene-related peptide in peripheral and central pain mechanisms including migraine. Pain (2017). https://doi.org/10.1097/j.pain.0000000000000831
2. F.A. Russell, R. King, S.J. Smillie, X. Kodji, S.D. Brain, Calcitonin gene-related peptide: physiology and pathophysiology. Physiol. Rev. (2014). https://doi.org/10.1152/physrev.00034.2013
3. A.J. Sorby-Adams, A.M. Marcoionni, E.R. Dempsey, J.A. Woenig, R.J. Turner, The role of neurogenic inflammation in blood-brain barrier disruption and development of cerebral oedema following acute central nervous system (CNS) injury. Int. J. Mol. Sci. (2017). https://doi.org/10.3390/ijms18081788
4. W.G. Lima, G.H. Marques-Oliveira, T.M. da Silva, V.E. Chaves, Role of calcitonin gene-related peptide in energy metabolism. Endocrine (2017). https://doi.org/10.1007/s12020-017-1404-4
5. D.L. Hay, D.R. Poyner, Calcitonin gene-related peptide, adrenomedullin and flushing. Maturitas (2009). https://doi.org/10.1016/j.maturitas.2009.08.011
6. S. Sharma, A. Mahajan, V.R. Tandon, Calcitonin gene-related peptide and menopause. J. Midlife. Health (2010). https://doi.org/10.4103/0976-7800.66985
7. D.W. Sturdee, M.S. Hunter, P.M. Maki, P. Gupta, J. Sassarini, J. C. Stevenson, M.A. Lumsden, The menopausal hot flush: a review. Climacteric (2017). https://doi.org/10.1080/13697137.2017.1306507
8. H.R. Morris, M. Panico, T. Etienne, J. Tippins, S.I. Girgis, I. MacIntyre, Isolation and characterization of human calcitonin gene-related peptide. Nature **308**, 746–748 (1984)
9. P.H. Steenbergh, J.W.M. Höppener, J. Zandberg, C.J.M. Lips, H. S. Jansz, A second human calcitonin/CGRP gene. FEBS Lett. (1985). https://doi.org/10.1016/0014-5793(85)80820-6
10. J.W. Hoppener, P.H. Steenbergh, J. Zandberg, A.H. Geurts van Kessel, S.B. Baylin, B.D. Nelkin, H.S. Jansz, C.J. Lips, The second human calcitonin/CGRP gene is located on chromosome 11. Hum. Genet. **70**, 259–263 (1985)
11. J.W. Hoppener, P.H. Steenbergh, J. Zandberg, E. Bakker, P.L. Pearson, A.H. Geurts van Kessel, H.S. Jansz, C.J. Lips, Localization of the polymorphic human calcitonin gene on chromosome 11. Hum. Genet. **66**, 309–312 (1984)
12. D. Van Rossum, U.K. Hanisch, R. Quirion, Neuroanatomical localization, pharmacological characterization and functions of CGRP, related peptides and their receptors. Neurosci. Biobehav. Rev. **21**, 649–678 (1997)
13. D.R. Poyner, P.M. Sexton, I. Marshall, D.M. Smith, R. Quirion, W. Born, R. Muff, J.A. Fischer, S.M. Foord, International Union of Pharmacology. XXXII. The mammalian calcitonin gene-related peptides, adrenomedullin, amylin, and calcitonin receptors. Pharmacol. Rev. (2002). https://doi.org/10.1124/pr.54.2.233
14. L.M. McLatchie, N.J. Fraser, M.J. Main, A. Wise, J. Brown, N. Thompson, R. Solari, M.G. Lee, S.M. Foord, RAMPs regulate the transport and ligand specificity of the calcitonin-receptor-like receptor. Nature **93**, 333–339 (1998)
15. S.P. Alexander, A.P. Davenport, E. Kelly, N. Marrion, J.A. Peters, H.E. Benson, E. Faccenda, A.J. Pawson, J.L. Sharman, C. Southan, J.A. Davies, The Concise Guide to

PHARMACOLOGY 2015/16: G protein-coupled receptors. Br. J. Pharmacol. (2015). https://doi.org/10.1111/bph.13348
16. C. Juaneda, Y. Dumont, R. Quirion, The molecular pharmacology of CGRP and related peptide receptor subtypes. Trends Pharmacol. Sci. (2000). https://doi.org/10.1016/S0165-6147(00)01555-8
17. Amgen Inc. Erenumab (Aimovig™): US prescribing information. https://www.accessdata.fda.gov/drugsatfdadocs/label/2018/761077s000lbl.pdf. (2018). Accessed 11 June 2018
18. A. Markham, Erenumab: first global approval. Drugs (2018). https://doi.org/10.1007/s40265-018-0944-0
19. ClinicalTrials.gov., Telcagepant for prevention of menstrually related migraine in female participants with episodic migraine (MK-0974-065). https://clinicaltrials.gov/ct2/show/NCT01125774?term=telcagepant&rank=1. (2018). Accessed 11 June 2018
20. M. Deen, E. Correnti, K. Kamm, T. Kelderman, L. Papetti, E. Rubio-Beltran, S. Vigneri, L. Edvinsson, A. Maassen Van Den Brink, Blocking CGRP in migraine patients - a review of pros and cons. J. Headache Pain (2017). https://doi.org/10.1186/s10194-017-0807-1
21. T.W. Ho, A.P. Ho, Y.J. Ge, C. Assaid, R. Gottwald, E.A. MacGregor, L.K. Mannix, W.P.J. van Oosterhout, J. Koppenhaver, C. Lines, M.D. Ferrari, D. Michelson, Randomized controlled trial of the CGRP receptor antagonist telcagepant for prevention of headache in women with perimenstrual migraine. Cephalalgia (2016). https://doi.org/10.1177/0333102415584308
22. D.J. Hewitt, S.K. Aurora, D.W. Dodick, P.J. Goadsby, Y.J. Ge, R. Bachman, D. Taraborelli, X. Fan, C. Assaid, C. Lines, T.W. Ho, Randomized controlled trial of the CGRP receptor antagonist MK-3207 in the acute treatment of migraine. Cephalalgia (2011). https://doi.org/10.1177/0333102411398399
23. H.-C. Diener, P. Barbanti, C. Dahlof, U. Reuter, J. Habeck, J. Podhorna, BI44370 TA, an oral CGRP antagonist for the treatment of acute migraine attacks: results from a phase II study. Cephalalgia (2011). https://doi.org/10.1177/0333102410388435
24. L. Kruger, P.W. Mantyh, C. Sternini, N.C. Brecha, C.R. Mantyh, Calcitonin gene-related peptide (CGRP) in the rat central nervous system: patterns of immunoreactivity and receptor binding sites. Brain Res. (1988). https://doi.org/10.1016/0006-8993(88)90395-2
25. P.M. Sexton, J.S. McKenzie, R.T. Mason, J.M. Moseley, T.J. Martin, F.A. Mendelsohn, Localization of binding sites for calcitonin gene-related peptide in rat brain by in vitro autoradiography. Neuroscience **19**, 1235–1245 (1986)
26. L. Edvinsson, E. Nilsson, I. Jansen-Olesen, Inhibitory effect of BIBN4096BS, CGRP(8-37), a CGRP antibody and an RNA-Spiegelmer on CGRP induced vasodilatation in the perfused and non-perfused rat middle cerebral artery. Br. J. Pharmacol. (2007). https://doi.org/10.1038/sj.bjp.0707134
27. D.L. Hay, M.L. Garelja, D.R. Poyner, C.S. Walker, Update on the pharmacology of calcitonin/CGRP family of peptides: IUPHAR Review 25. Br. J. Pharmacol. (2018). https://doi.org/10.1111/bph.14075
28. H. Yoshizaki, M. Takamiya, T. Okada, Characterization of picomolar affinity binding sites for [125I]-human calcitonin gene-related peptide in rat brain and heart. Biochem. Biophys. Res. Commun. **146**, 443–451 (1987)
29. T. Dennis, A. Fournier, A. Cadieux, F. Pomerleau, F.B. Jolicoeur, S. St Pierre, R. Quirion, hCGRP8-37, a calcitonin gene-related peptide antagonist revealing calcitonin gene-related peptide receptor heterogeneity in brain and periphery. J. Pharmacol. Exp. Ther. 254, 123–128 (1990)
30. H. Nakamuta, Y. Fukuda, M. Koida, N. Fujii, A. Otaka, S. Funakoskhi, H. Yajima, N. Mitsuyasu, R.C. Orlowshi, Binding sites of calcitonin gene-related peptide (CGRP): abundant occurrence in visceral organs. Jpn. J. Pharmacol. **42**, 175–180 (1986)
31. M. Roa, J.P. Changeux, Characterization and developmental evolution of a high-affinity binding site for calcitonin gene-related peptide on chick skeletal muscle membrane. Neuroscience (1991). https://doi.org/10.1016/0306-4522(91)90349-S
32. C.E. Hill, D.J. Gould, J. Strigas, E. Burcher, M. Vidovic, Sensory nerves play an efferent role in the function of the arterioles, but not the dilator muscle, of the rat iris. J. Auton. Nerv. Syst. **58**, 89–100 (1996)
33. J.C.W. Mak, P.J. Barnes, Autoradiographic localization of calcitonin gene-related peptide (CGRP) binding sites in human and guinea pig lung. Peptides 9, 957–963 (1988)
34. J.P. McGillis, S. Humphreys, V. Rangnekar, J. Ciallella, Modulation of B lymphocyte differentiation by calcitonin gene-related peptide (CGRP). I. Characterization of high-affinity CGRP receptors on murine 70Z/3 cells. Cell. Immunol. **150**, 391–404 (1993)
35. A.M. Salmon, M.I. Damaj, L.M. Marubio, M.P. Epping-Jordan, E. Merlo-Pich, J.P. Changeux, Altered neuroadaptation in opiate dependence and neurogenic inflammatory nociception in alpha CGRP-deficient mice. Nat. Neurosci. (2001). https://doi.org/10.1038/86001
36. K. Takami, Y. Kawai, S. Uchida, M. Tohyama, Y. Shiotani, H. Yoshida, P.C. Emson, S. Girgis, C.J. Hillyard, I. MacIntyre, Effect of calcitonin gene-related peptide on contraction of striated muscle in the mouse. Neurosci. Lett. **60**, 227–230 (1985)
37. S. Uchida, H. Yamamoto, S. Lio, N. Matsumoto, X.B. Wang, N. Yonehara, Y. Imai, R. Inoki, H. Yoshida, Release of calcitonin gene-related peptide-like immunoreactive substance from neuromuscular junction by nerve excitation and its action on striated muscle. J. Neurochem. **54**, 1000–1003 (1990)
38. C. Gennari, J.A. Fischer, Cardiovascular action of calcitonin gene-related peptide in humans. Calcif. Tissue Int. (1985). https://doi.org/10.1007/BF02554909
39. M.D. Harzenetter, A.R. Novotny, P. Gais, C.A. Molina, F. Altmayr, B. Holzmann, Negative regulation of TLR responses by the neuropeptide CGRP is mediated by the transcriptional repressor ICER. J. Immunol. (2007). https://doi.org/10.4049/jimmunol.179.1.607
40. E. Tanaka, S. Uchiyama, S. Nakano, Effects of calcitonin gene-related peptide and vasoactive intestinal peptide on nicotine-induced sweating in man. J. Auton. Nerv. Syst. **30**, 265–268 (1990)
41. J. Machado, L.H. Manfredi, W.A. Silveira, D.A.P. Goncalves, D. Lustrino, N.M. Zanon, I.C. Kettelhut, L.C. Navegantes, Calcitonin gene-related peptide inhibits autophagic-lysosomal proteolysis through cAMP/PKA signaling in rat skeletal muscles. Int. J. Biochem. Cell Biol. (2016). https://doi.org/10.1016/j.biocel.2015.12.011
42. C.S. Walker, D.L. Hay, S.M. Fitzpatrick, G.J.S. Cooper, K.M. Loomes, alpha-Calcitonin gene related peptide (alpha-CGRP) mediated lipid mobilization in 3T3-L1 adipocytes. Peptides. (2014). https://doi.org/10.1016/j.peptides.2014.05.011
43. T. Schinke, S. Liese, M. Priemel, M. Haberland, A.F. Schilling, P. Catala-Lehnen, D. Blicharski, J.M. Rueger, R.F. Gagel, R.B. Emeson, M. Amling, Decreased bone formation and osteopenia in mice lacking alpha-calcitonin gene-related peptide. J. Bone Miner. Res. (2004). https://doi.org/10.1359/JBMR.040915
44. N. Takahashi, Y. Matsuda, K. Sato, P.R. de Jong, S. Bertin, K. Tabeta, K. Yamazaki, Neuronal TRPV1 activation regulates alveolar bone resorption by suppressing osteoclastogenesis via CGRP. Sci. Rep. (2016). https://doi.org/10.1038/srep29294
45. S.D. Brain, T.J. Williams, J.R. Tippins, H.R. Morris, I. MacIntyre, Calcitonin gene-related peptide is a potent vasodilator. Nature (1985). https://doi.org/10.1038/313054a0

46. S.D. Brain, Vascular actions of calcitonin gene-related peptide and adrenomedullin. Physiol. Rev. (2004). https://doi.org/10.1152/physrev.00037.2003
47. R. Uddman, L. Edvinsson, E. Ekblad, R. Hakanson, F. Sundler, Calcitonin gene-related peptide (CGRP): perivascular distribution and vasodilatory effects. Regul. Pept. **15**, 1–23 (1986)
48. K. Kumazawa, G. Sobue, T. Mitsuma, T. Ogawa, Modulatory effects of calcitonin gene-related peptide and substance P on human cholinergic sweat secretion. Clin. Auton. Res. (1994). https://doi.org/10.1007/BF01821532
49. T. Schlereth, J.O. Dittmar, B. Seewald, F. Birklein, Peripheral amplification of sweating – a role for calcitonin gene-related peptide. J. Physiol. (2006). https://doi.org/10.1113/jphysiol.2006.116111
50. T. Hasegawa, K. Yokotani, Y. Okuma, M. Manabe, M. Hirakawa, Y. Osumi, Microinjection of $f\alpha$-calcitonin gene-related peptide into the hypothalamus activates sympathetic outflow in rats. J. Pharmacol. **61**, 325–332 (1993)
51. Research on the menopause in the 1990s. Report of a WHO Scientific Group (WHO, Switzerland, 1996)
52. Y. Handelsman, J.I. Mechanick, S. Dagogo-Jack, J.A. Davidson, AACE Guidelines. Endocr. Pract. (2011). https://doi.org/10.4158/EP.17.S2.1
53. P.K. Dalal, M. Agarwal, Postmenopausal syndrome. Indian J. Psychiatry. (2015). https://doi.org/10.4103/0019-5545.161483
54. J.T. Bromberger, K.A. Matthews, L.H. Kuller, R.R. Wing, E.N. Meilahn, P. Plantinga, Prospective study of the determinants of age at menopause. Am. J. Epidemiol. (1997). https://doi.org/10.1093/oxfordjournals.aje.a009083
55. T. Okeke, U. Anyaehie, C. Ezenyeaku, Premature menopause. Ann. Med. Health Sci. Res. (2013). https://doi.org/10.4103/2141-9248.109458
56. B.K. Jacobsen, I. Heuch, G. Kvale, Age at natural menopause and all-cause mortality: a 37-year follow-up of 19,731 Norwegian women. Am. J. Epidemiol. (2003). https://doi.org/10.1093/aje/kwg066
57. R. Cui, H. Iso, H. Toyoshima, C. Date, A. Yamamoto, S. Kikuchi, T. Kondo, Y. Watanabe, A. Koizumi, Y. Inaba, A. Tamakoshi, Relationships of age at menarche and menopause, and reproductive year with mortality from cardiovascular disease in Japanese postmenopausal women: the JACC study. J. Epidemiol. **16**, 177–184 (2006)
58. J. Kozakowski, M. Gietka-Czernel, D. Leszczynska, A. Majos, Obesity in menopause - our negligence or an unfortunate inevitability?. Menopause Rev. (2017). https://doi.org/10.5114/pm.2017.68594
59. R. Bijelic, S. Milicevic, J. Balaban, Risk factors for osteoporosis in postmenopausal women. Med. Arch. (2017). https://doi.org/10.5455/medarh.2017.71.25-28
60. B.E. Bernstein, T.S. Mikkelsen, X. Xie, M. Kamal, D.J. Huebert, J. Cuff, B. Fry, A. Meissner, M. Wernig, M., K. Plath, R. Jaenisch, A. Wagschal, R. Feil, S.L. Schreiber, E.S. Lander, A bivalent chromatin structure marks key developmental genes in embryonic stem cells. Cell (2006). https://doi.org/10.1016/j.cell.2006.02.041
61. D. Kritz-Silverstein, E. Barrett-Connor, Early menopause, number of reproductive years, and bone mineral density in postmenopausal women. Am. J. Public Health **83**, 983–988 (1993)
62. ACOG Practice Bulletin No. 141: management of menopausal symptoms. Obstet. Gynecol. (2014). https://doi.org/10.1097/01.AOG.0000441353.20693.78
63. I. Goranitis, L. Bellanca, A.J. Daley, A. Thomas, H. Stokes-Lampard, A.K. Roalfe, S. Jowett, Aerobic exercise for vasomotor menopausal symptoms: a cost-utility analysis based on the Active Women trial. PLoS One (2017). https://doi.org/10.1371/journal.pone.0184328
64. R.W. Rebar, I.B. Spitzer, The physiology and measurement of hot flushes. Am. J. Obstet. Gynecol. (1987). https://doi.org/10.1016/0002-9378(87)90165-7
65. C.M. Estrada, V. Ghisays, E.T. Nguyen, J.L. Caldwell, J. Streicher, M.B. Solomon, Estrogen signaling in the medial amygdala decreases emotional stress responses and obesity in ovariectomized rats. Horm. Behav. (2018). https://doi.org/10.1016/j.yhbeh.2017.12.002
66. R.R. Freedman, Imaging and menopausal hot flashes. Temperature (Austin.) (2016). https://doi.org/10.1080/23328940.2016.1208318
67. P.K.H. Morrow, D.N. Mattair, G.N. Hortobagyi, Hot flashes: a review of pathophysiology and treatment modalities. Oncologist (2011). https://doi.org/10.1634/theoncologist.2011-0174
68. A.A. Krull, S.A. Larsen, D.K. Clifton, G. Neal-Perry, R.A. Steiner, A. comprehensive method to quantify adaptations by male and female mice with hot flashes induced by the neurokinin B receptor agonist senktide. Endocrinology (2017). https://doi.org/10.1210/en.2017-00142
69. G.W. Molnar, Body temperatures during menopausal hot flashes. J. Appl. Physiol. (1975). https://doi.org/10.1152/jappl.1975.38.3.499
70. S. Valdemarsson, L. Edvinsson, P. Hedner, R. Ekman, Hormonal influence on calcitonin gene-related peptide in man: effects of sex difference and contraceptive pills. Scand. J. Clin. Lab. Invest. (1990). https://doi.org/10.3109/00365519009091595
71. A. Valentini, F. Petraglia, D. De Vita, C. Nappi, A. Margutti, E. C. Degli Uberti, A.R. Genazzani, Changes of plasma calcitonin gene-related peptide levels in postmenopausal women. Am. J. Obstet. Gynecol. (1996). https://doi.org/10.1053/ob.1996.v175.a74287
72. Y.A. Wyon, A.C. Spetz, G.E. Theodorsson, M.L. Hammar, Concentrations of calcitonin gene-related peptide and neuropeptide Y in plasma increase during flushes in postmenopausal women. Menopause (2000). https://doi.org/10.1097/00042192-200007010-00005
73. J.T. Chen, M. Shiraki, Menopausal hot flash and calcitonin gene-related peptide; effect of Keishi-bukuryo-gan, a kampo medicine, related to plasma calciotonin gene-related peptide level. Maturitas (2003). https://doi.org/10.1016/S0378-5122(03)00128-2
74. P. Gupta, A. Harte, D.W. Sturdee, A. Sharma, A.H. Barnett, S. Kumar, P.G. McTernan, Effects of menopausal status on circulating calcitonin gene-related peptide and adipokines: implications for insulin resistance and cardiovascular risks. Climacteric (2008). https://doi.org/10.1080/13697130802378493
75. Y. Wyon, J. Frisk, T. Lundeberg, E. Theodorsson, M. Hammar, Postmenopausal women with vasomotor symptoms have increased urinary excretion of calcitonin gene-related peptide. Maturitas **30**, 289–294 (1998)
76. A.C. Spetz, B. Pettersson, E. Varenhorst, E. Theodorsson, L.H. Thorell, M Hammar, Momentary increase in plasma calcitonin gene-related peptide is involved in hot flashes in men treated with castration for carcinoma of the prostate. J Urol. (2001). http://www.ncbi.nlm.nih.gov/pubmed/11586209
77. A.C. Holm, L.H. Thorell, E. Theodorsson, M. Hammar, Hot flushes in healthy aging men differ from those in men with prostate cancer and in menopausal women. Gynecol. Endocrinol. (2012). https://doi.org/10.3109/09513590.2011.588744
78. P.R.R. Gangula, M. Chauhan, L. Reed, C. Yallampalli, Age-related changes in dorsal root ganglia, circulating and vascular calcitonin gene-related peptide (CGRP) concentrations in female rats: effect of female sex steroid hormones, Neurosci Lett. (2009). https://doi.org/10.1016/j.neulet.2009.02.068

79. M. Noguchi, Y. Ikarashi, M. Yuzurihara, K. Mizoguchi, K. Kurauchi, J. Chen, A. Ishige, Up-regulation of calcitonin gene-related peptide receptors underlying elevation of skin temperature in ovariectomized rats. J. Endocrinol. **175**, 177–183 (2002)
80. A. Spinetti, A. Margutti, S. Bertolini, F. Bernardi, G. BiFulco, E. C. Degli Uberti, F. Petraglia, A.R. Genazzani, Hormonal replacement therapy affects calcitonin gene-related peptide and atrial natriuretic peptide secretion in postmenopausal women. Eur. J. Endocrinol. **137**, 664–669 (1997)
81. P.R.R. Gangula, S.J. Wimalawansa, C. Yallampalli, Pregnancy and sex steroid hormones enhance circulating calcitonin gene-related peptide concentrations in rats. Hum. Reprod. (2000). http://www.ncbi.nlm.nih.gov/pubmed/10739848
82. M. Noguchi, Y. Ikarashi, M. Yuzurihara, Y. Kase, S. Takeda, M. Aburada, Effects of 17 beta-estradiol and the Japanese herbal medicine Keishi-bukuryo-gan on the release and synthesis of calcitonin gene-related peptide in ovariectomized rats. J. Pharmacol. Sci. (2003). https://doi.org/10.1254/jphs.93.80
83. C.N. Mowa, S. Usip, J. Collins, M. Storey-Workley, K.M. Hargreaves, R.E. Papka, The effects of pregnancy and estrogen on the expression of calcitonin gene-related peptide (CGRP) in the uterine cervix, dorsal root ganglia and spinal cord. Peptides (2003). https://doi.org/10.1016/j.peptides.2003.07.009
84. G. Gon, A. Giaid, J.H. Steel, D.J.O. Halloran, S.V.A.N. Noorden, M.A. Ghatei, P.M. Jones, G. Susan, S.R. Bloom, J.M. Polak, Localization of immunoreactivity for calcitonin gene-related peptide in the rat anterior pituitary during ontogeny and gonadal steroid manipulations and detection of its messenger ribonucleid acid. Endocrinology **127**, 2618–2629 (1990)
85. Y. Lamari, M. Ghorbel, J.M. Garel, Effects of 17 beta-estradiol on calcitonin-gene-related peptide secretions and contents in a murine medullary thyroid carcinoma C-cell line (CA-77). Reprod. Nutr. Dev. **35**, 655–661 (1995)
86. V. Pota, V. Quagliariello, E. Armenia, C. Aurilio, M.B. Passavanti, P. Sansone, M. Iannotti, M. Catauro, S. Coaccioli, M. Barbarisi, M.C. Pace, CGRP and visceral pain: the role of sex hormones in in vitro experiment. J. Cell. Biochem. (2017). https://doi.org/10.1002/jcb.25680
87. M. Aggarwal, V. Puri, S. Puri, Effects of estrogen on the serotonergic system and calcitonin generelated peptide in trigeminal ganglia of rats. Ann. Neurosci. (2012). https://doi.org/10.5214/ans.0972.7531.190403
88. R.T. Dos Santos Pereira, C.S. Porto, F.M.F. Abdalla, Ovariectomy and 17beta-estradiol replacement play a role on the expression of Endonuclease-G and phosphorylated cyclic AMP response element-binding (CREB) protein in hippocampus. Mol. Cell. Endocrinol. (2014). https://doi.org/10.1016/j.mce.2013.09.037
89. L. Yang, Q.-G. Zhang, C. Zhou, F. Yang, Y. Zhang, R. Wang, D. W. Brann, Extranuclear estrogen receptors mediate the neuroprotective effects of estrogen in the rat hippocampus. PLoS One (2010). https://doi.org/10.1371/journal.pone.0009851
90. S.M. Aronica, W.L. Kraus, B.S. Katzenellenbogen, Estrogen action via the cAMP signaling pathway: stimulation of adenylate cyclase and cAMP-regulated gene transcription. Proc. Natl. Acad. Sci. USA **91**, 8517–8521 (1994)
91. T.D. Purves-Tyson, J.R. Keast, Rapid actions of estradiol on cyclic amp response-element binding protein phosphorylation in dorsal root ganglion neurons. Neuroscience (2004). https://doi.org/10.1016/j.neuroscience.2004.08.019
92. M. Genua, G. Pandini, D. Sisci, G. Castoria, M. Maggiolini, R. Vigneri, A. Belfiore, Role of cyclic AMP response element-binding protein in insulin-like growth factor-i receptor up-regulation by sex steroids in prostate cancer cells. Cancer Res. (2009). https://doi.org/10.1158/0008-5472.CAN-09-0088
93. K. Freeland, Y.Z. Liu, D.S. Latchman, Distinct signalling pathways mediate the cAMP response element (CRE)-dependent activation of the calcitonin gene-related peptide gene promoter by cAMP and nerve growth factor. Biochem. J. **345**(Pt 2), 233–238 (2000)
94. C.S. Walker, X. Li, L. Whiting, S. Glyn-Jones, S. Zhang, A.J. Hickey, M.A. Sewell, K. Ruggiero, A.R.J.Phillips, E.W. Kraegen, D.L. Hay, G.J.S.., Cooper, K.M. Loomes, Mice lacking the neuropeptide alpha-calcitonin gene-related peptide are protected against diet-induced obesity. Endocrinology. (2010). https://doi.org/10.1210/en.2010-0284
95. T. Kobayashi, O. Ushijima, J.T. Chen, M. Shiraki, T. Ohta, M. Kiyoki, Basal tail skin temperature elevation and augmented response to calcitonin gene-related peptide in ovariectomized rats. J. Endocrinol. (1995). https://doi.org/10.1677/joe.0.1460431
96. M. Noguchi, Y. Ikarashi, M. Yuzurihara, Y. Kase, S. Takeda, M. Aburada, Significance of measured elevation of skin temperature induced by calcitonin gene-related peptide in anaesthetized rats. J. Pharm. Pharmacol. (2003). https://doi.org/10.1211/0022357022142
97. M. Noguchi, M. Yuzurihara, Y. Ikarashi, Effects of the vasoactive neuropeptides calcitonin gene-related peptide, substance P and vasoactive intestinal polypeptide on skin temperature in ovariectomized rats. Neuropeptides (2002). https://doi.org/10.1016/S0143-4179(02)00090-2
98. M. Noguchi, Y. Ikarashi, M. Yuzurihara, Y. Kase, K. Watanabe, G.A. Plotnikoff, S. Takeda, M. Aburada, Skin temperature rise induced by calcitonin gene-related peptide in gonadotropin-releasing hormone analogue-treated female rats and alleviation by Keishi-bukuryo-gan, a Japanese herbal medicine. Life Sci. (2005). https://doi.org/10.1016/j.lfs.2004.09.032
99. M. Yuzurihara, Y. Ikarashi, M. Noguchi, Y. Kase, S. Takeda, M. Aburada, Prevention by 17β-estradiol and progesterone of calcitonin gene-related peptide-induced elevation of skin temperature in castrated male rats. Urology (2004). https://doi.org/10.1016/j.urology.2004.06.013
100. P. Holzer, Peptidergic sensory neurons in the control of vascular functions: mechanisms and significance in the cutaneous and splanchnic vascular beds, Rev. Physiol. Biochem. Pharmacol. (1992). https://doi.org/10.1007/BFb0033194
101. D. Roosterman, T. Goerge, S.W. Schneider, N.W. Bunnett, M. Steinhoff, Neuronal control of skin function: the skin as a neuroimmunoendocrine organ. Physiol. Rev. (2006). https://doi.org/10.1152/physrev.00026.2005
102. S.R. Hughes, S.D. Brain, A calcitonin gene-related peptide (CGRP) antagonist (CGRP8-37) inhibits microvascular responses induced by CGRP and capsaicin in skin. Br. J. Pharmacol. **104**, 738–742 (1991)
103. K.J. Escott, S.D. Brain, Effect of a calcitonin gene-related peptide antagonist (CGRP8-37) on skin vasodilatation and oedema induced by stimulation of the rat saphenous nerve. Br. J. Pharmacol. **110**, 772–776 (1993)
104. K.J. Escott, D.T. Beattie, H.E. Connor, S.D. Brain, Trigeminal ganglion stimulation increases facial skin blood flow in the rat: a major role for calcitonin gene-related peptide. Brain Res. **669**, 93–99 (1995)
105. C.A. Salvatore, E.L. Moore, A. Calamari, J.J. Cook, M.S. Michener, S. O'Malley, P.J. Miller, C. Sur, D.L.J. Williams, Z. Zeng, A. Danziger, J.J. Lynch, C.P. Regan, J.F. Fay, Y.S. Tang, C.-C. Li, N.T. Pudvah, R.B. White, I.M. Bell, S.N. Gallicchio, S. L. Graham, H.G. Selnick, J.P. Vacca, S.A. Kane, Pharmacological properties of MK-3207, a potent and orally active calcitonin gene-related peptide receptor antagonist. J. Pharmacol. Exp. Ther. (2010). https://doi.org/10.1124/jpet.109.163816
106. S.R. Sinclair, S. A. Kane, B.J. Van der Schueren, A. Xiao, K.J. Willson, J. Boyle, I. de Lepeleire, Y. Xu, L. Hickey, W.S. Denney, C.-C. Li, J. Palcza, F.H.M. Vanmolkot, M. Depre, A. Van Hecken, M.G. Murphy, T.W. Ho, J.N. de Hoon, Inhibition

of capsaicin-induced increase in dermal blood flow by the oral CGRP receptor antagonist, telcagepant (MK-0974). Br. J. Clin. Pharmacol. (2010). https://doi.org/10.1111/j.1365-2125.2009.03543.x
107. C.-C. Li, S. Vermeersch, W.S. Denney, W. P. Kennedy, J. Palcza, A. Gipson, T.H. Han, R. Blanchard, I. De Lepeleire, M. Depre, M.G. Murphy, K. Van Dyck, J.N. de Hoon, JCharacterizing the PK/PD relationship for inhibition of capsaicin-induced dermal vasodilatation by MK-3207, an oral calcitonin gene related peptide receptor antagonist. Br. J. Clin. Pharmacol. (2015). https://doi.org/10.1111/bcp.12547
108. T. Vu, P. Ma, J.S. Chen, J. de Hoon, A. Van Hecken, L. Yan, L. S. Wu, L. Hamilton, G. Vargas, Pharmacokinetic-pharmacodynamic relationship of erenumab (AMG 334) and capsaicin-induced dermal blood flow in healthy and migraine subjects. Pharm. Res. (2017). https://doi.org/10.1007/s11095-017-2183-6
109. B.J. Van der Schueren, A. Rogiers, F.H. Vanmolkot, A. Van Hecken, M. Depre, S.A. Kane, I. De Lepeleire, S.R. Sinclair, J. N. de Hoon, Calcitonin gene-related peptide8-37 antagonizes capsaicin-induced vasodilation in the skin: evaluation of a human in vivo pharmacodynamic model. J. Pharmacol. Exp. Ther. (2008). https://doi.org/10.1124/jpet.107.133868
110. H. Minato, A. Ikeno, N. Watanabe, J.Tsuji, Effect of OS-0689, a novel SERM, on periarterial nerve function in tail arteries of ovariectomized rats. Maturitas (2005). https://doi.org/10.1016/j.maturitas.2004.11.003
111. S.P. Han, L. Naes, T.C. Westfall, Inhibition of periarterial nerve stimulation-induced vasodilation of the mesenteric arterial bed by CGRP (8-37) and CGRP receptor desensitization. Biochem. Biophys. Res. Commun. **168**, 786–791 (1990)
112. Y. Oh-hashi, T. Shindo, Y. Kurihara, T. Imai, Y. Wang, H. Morita, Y. Imai, Y. Kayaba, H. Nishimatsu, Y. Suematsu, Y. Hirata, Y. Yazaki, R. Nagai, T. Kuwaki, H. Kurihara, Elevated sympathetic nervous activity in mice deficient in alphaCGRP. Circ. Res. (2001). https://doi.org/10.1161/hh2301.100812
113. P.R. Gangula, H. Zhao, S.C. Supowit, S.J. Wimalawansa, D.J. Dipette, K.N. Westlund, R.F. Gagel, C. Yallampalli, Increased blood pressure in alpha-calcitonin gene-related peptide/calcitonin gene knockout mice.Hypertension **35**, 470–475 (2000).
114. J. Jernbeck, M. Edner, C.J. Dalsgaard, B. Pernow, The effect of calcitonin gene-related peptide (CGRP) on human forearm blood flow. Clin. Physiol. **10**, 335–343 (1990)
115. K. Jager, R. Muench, H. Seifert, C. Beglinger, A. Bollinger, J.A. Fischer, Calcitonin gene-related peptide (CGRP) causes redistribution of blood flow in humans. Eur. J. Clin. Pharmacol. **39**, 491–494 (1990)
116. S.C. Landis, J.R. Fredieu, Coexistence of calcitonin gene-related peptide and vasoactive intestinal peptide in cholinergic sympathetic innervation of rat sweat glands. Brain Res. **377**, 177–181 (1986)
117. N. Charkoudian, E.C.J. Hart, J.N. Barnes, M.J. Joyner, Autonomic control of body temperature and blood pressure: influences of female sex hormones. Clin. Auton. Res. (2017). https://doi.org/10.1007/s10286-017-0420-z
118. C.J. Smith, J.M. Johnson, Responses to hyperthermia. Optimizing heat dissipation by convection and evaporation: neural control of skin blood flow and sweating in humans. Auton. Neurosci. (2016). https://doi.org/10.1016/j.autneu.2016.01.002
119. R.R. Freedman, W. Krell, Reduced thermoregulatory null zone in postmenopausal women with hot flashes. Am. J. Obstet. Gynecol. (1999). https://doi.org/10.1016/S0002-9378(99)70437-0
120. A.E. Herbison, Identification of a sexually dimorphic neural population immunoreactive for calcitonin gene-related peptide (CGRP) in the rat medial preoptic area. Brain Res. **591**, 289–295 (1992)
121. A.E. Herbison, S. Dye, Perinatal and adult factors responsible for the sexually dimorphic calcitonin gene-related peptide-containing cell population in the rat preoptic area. Neuroscience (1993). https://doi.org/10.1016/0306-4522(93)90590-C
122. D.C. Braasch, E.M. Deegan, E.R. Grimm, J.D. Griffin, Calcitonin gene-related peptide alters the firing rates of hypothalamic temperature sensitive and insensitive neurons. BMC Neurosci. (2008). https://doi.org/10.1186/1471-2202-9-64
123. A. Kobayashi, T. Osaka, Y. Namba, S. Inoue, S. Kimura, CGRP microinjection into the ventromedial or dorsomedial hypothalamic nucleus activates heat production. Brain Res. (1999). https://doi.org/10.1016/S0006-8993(99)01333-5
124. R.R. Freedman, Menopausal hot flashes: mechanisms, endocrinology, treatment. J. Steroid Biochem. Mol. Biol. (2014). https://doi.org/10.1016/j.jsbmb.2013.08.010
125. C.U. Pae, M.H. Park, D.M. Marks, C. Han, A.A. Patkar, P.S. Masand, Desvenlafaxine, a serotonin-norepinephrine uptake inhibitor for major depressive disorder, neuropathic pain and the vasomotor symptoms associated with menopause. Curr. Opin. Investig. Drugs **10**, 75–90 (2009)
126. S.F. Morrison, Central control of body temperature. F1000Res (2016). https://doi.org/10.12688/f1000research.7958.1
127. Y. Hu, C. Converse, M.C. Lyons, W.H. Hsu, Neural control of sweat secretion: a review. Br. J. Dermatol. (2017). https://doi.org/10.1111/bjd.15808
128. K.F. Malik, H.H. Feder, J.I. Morrell, Estrogen receptor immunostaining in the preoptic area and medial basal hypothalamus of estradiol benzoate- and prazosin-treated female guinea-pigs. J. Neuroendocrinol. **5**, 297–306 (1993)
129. J.A. Butler, I. Kallo, M. Sjoberg, C.W. Coen, Evidence for extensive distribution of oestrogen receptor alpha-immunoreactivity in the cerebral cortex of adult rats. J. Neuroendocrinol. **11**, 325–329 (1999)
130. R.R. Freedman, M.D. Benton, R.J. Genik 2nd, F.X. Graydon, Cortical activation during menopausal hot flashes. Fertil. Steril. (2006). https://doi.org/10.1016/j.fertnstert.2005.08.026
131. D.F. Archer, D.W. Sturdee, R. Baber, T.J. de Villiers, A. Pines, R.R. Freedman, A. Gompel, M. Hickey, M.S. Hunter, R.A. Lobo, M.A. Lumsden, A.H. MacLennan, P. Maki, S. Palacios, D. Shah, P. Villaseca, M. Warren, Menopausal hot flushes and night sweats: where are we now?. Climacteric (2011). https://doi.org/10.3109/13697137.2011.608596
132. O.H. Franco, T. Muka, V. Colpani, S. Kunutsor, S. Chowdhury, R. Chowdhury, M. Kavousi, Vasomotor symptoms in women and cardiovascular risk markers: systematic review and meta-analysis. Maturitas (2015). https://doi.org/10.1016/j.maturitas.2015.04.016
133. X. Wu, J.-T.Zhang, J. Liu, S.Yang, T. Chen, J.-G. Chen, F. Wang, Calcitonin gene-related peptide erases the fear memory and facilitates long-term potentiation in the central nucleus of the amygdala in rats. J. Neurochem. (2015). https://doi.org/10.1111/jnc.13246
134. J.S. Han, H. Adwanikar, Z. Li, G. Ji, V. Neugebauer, Facilitation of synaptic transmission and pain responses by CGRP in the amygdala of normal rats. Mol. Pain. (2010). https://doi.org/10.1186/1744-8069-6-10
135. A.Y. Deutch, R.H. Roth, Calcitonin gene-related peptide in the ventral tegmental area: selective modulation of prefrontal cortical dopamine metabolism. Neurosci. Lett. **74**, 169–174 (1987)
136. A.A. Mathe, P. Hertel, G.G. Nomikos, S. Gruber, J.M. Mathe, T. H. Svensson, The psychotomimetic drugs D-amphetamine and phencyclidine release calcitonin gene-related peptide in the limbic forebrain of the rat. J. Neurosci. Res. **46**, 316–323 (1996)