Title
Long-term ionic plasticity of GABAergic signalling in the hypothalamus

Journal
Journal of Neuroendocrinology, 31(8)

ISSN
0953-8194

Authors
Kim, Young-Beom
Colwell, Christopher S
Kim, Yang In

Publication Date
2019-08-01

DOI
10.1111/jne.12753

Peer reviewed

Received: 14 March 2019 | Revised: 24 May 2019 | Accepted: 3 June 2019

DOI: 10.1111/jne.12753

EDITOR INVITED REVIEW

Journal of Neuroendocrinology WILEY

Long-term ionic plasticity of GABAergic signalling in the hypothalamus

Young-Beom Kim[1,2] | Christopher S. Colwell[3] | Yang In Kim[1,2]

[1]Department of Physiology, Korea University College of Medicine, Seoul, Korea

[2]Neuroscience Research Institute, Korea University, Seoul, Korea

[3]Department of Psychiatry and Biobehavioral Sciences, UCLA School of Medicine, Los Angeles, CA, USA

Correspondence
Yang In Kim, Department of Physiology, Korea University College of Medicine, Seoul, Korea.

Christopher S. Colwell, Department of Psychiatry and Biobehavioral Sciences, UCLA School of Medicine, Los Angeles, CA, USA.
Emails: yikim@korea.ac.kr (YIK) and ccolwell@mednet.ucla.edu (CSC)

Funding information
National Research Foundation of Korea (NRF) grants funded by the Korea government (MSIP), Grant/Award Number: 2017R1A2B2002277 and 2019R1I1A1A01057744; Korea University

Abstract

The hypothalamus contains a number of nuclei that subserve a variety of functions, including generation of circadian rhythms, regulation of hormone secretion and maintenance of homeostatic levels for a variety of physiological parameters. Within the hypothalamus, γ-amino-butyric acid (GABA) is one of the major neurotransmitters responsible for cellular communication. Although GABA most commonly serves as an inhibitory neurotransmitter, a growing body of evidence indicates that it can evoke post-synaptic excitation as a result of the active regulation of intracellular chloride concentration. In this review, we consider the evidence for this ionic plasticity of GABAergic synaptic transmission in five distinct cases in hypothalamic cell populations. We argue that this plasticity serves as part of the functional response to or is at least associated with dehydration, lactation, hypertension and stress. As such, GABA excitation should be considered as part of the core homeostatic mechanisms of the hypothalamus.

KEYWORDS
chloride, GABA, hypothalamus, KCC2, NKCC1

1 | INTRODUCTION

The hypothalamus contains a number of small nuclei with a variety of functions, including generation of circadian rhythms, regulation of hormone secretion and maintenance of homeostatic levels for a variety of physiological parameters. One of the critical functions of the hypothalamus is to serve as the central interface of the nervous and endocrine systems. These functions require coordination between the cell populations within the hypothalamus, as well as interconnections of the hypothalamus with other parts of the central nervous system (CNS). As in other regions of the CNS, one of the major neurotransmitters responsible for synaptic communication in the hypothalamus is γ-amino-butyric acid (GABA).[1] Although GABA is widely accepted as an inhibitory neurotransmitter, a growing body of evidence indicates that it can function as an excitatory neurotransmitter in hypothalamic neurones in adulthood, often in response to physiological demands.[2,3] Earlier studies have shown that GABA acts as an excitatory neurotransmitter in immature CNS neurones at early developmental stages to promote neuronal maturation and neural networking and, in some mature CNS neurones, it is implicated in the genesis and/or maintenance of certain pathological conditions such as epilepsy and neuropathy.[4] Moreover, extensive work in gonadotrophin-releasing hormone (GnRH) neurones has shown that, in at least a subpopulation of these neurones, synaptic and exogenous agonist-induced activation of $GABA_A$ receptors leads to depolarisation and action potential firing,[5] although long-term ionic plasticity in $GABA_A$ receptor-mediated transmission ($GABA_A$ signalling) has not been reported in GnRH neurones. In this review, we summarise the results of previous studies on the long-term plasticity of $GABA_A$ signalling in hypothalamic neurones dependent on the modulation of postsynaptic $[Cl^-]_i$. Plasticity in $GABA_A$ receptor subunit composition and/or other potential forms of plasticity such as that of presynaptic GABA release are not considered in this review.

2 | SOME BASICS ON $GABA_A$ SIGNALLING

The $GABA_A$ receptor is a ligand-gated ion channel permeable in both directions to monovalent anions, Cl^- and HCO_3^-.[6,7] Hence, when $GABA_A$ receptor is activated, both Cl^- and HCO_3^- can flow through open $GABA_A$ receptor channel; the size and direction of Cl^- and HCO_3^- currents through this channel are determined by electrochemical gradients for these ions. In CNS neurones, the Cl^- equilibrium potential (E_{Cl}) and HCO_3^- equilibrium potential (E_{HCO3}) are usually set around −90 mV and −10 mV, respectively, and the equilibrium potential of $GABA_A$ receptor-mediated current/potential (E_{GABA}) is set between the E_{Cl} and E_{HCO3}, although closer to the E_{Cl}. This is because $GABA_A$ receptor channel is 2.5-5 times more permeable to Cl^- than HCO_3^-.[8,9]

In mammalian CNS neurones, changes in $[HCO_3^-]_i$, which is set by a pH-regulatory mechanism,[6] can alter the E_{GABA}. Likewise, changes in $[Cl^-]_i$ can reset the E_{GABA}. The $[Cl^-]_i$ in these cells is determined not only by the flux of Cl^- through $GABA_A$ receptor channels, but also by the action of the cation chloride cotransporters (CCCs) Na^+-K^+-$2Cl^-$ cotransporter 1 (NKCC1) and K^+-Cl^- cotransporter 2 (KCC2).[4,10,11] These are secondary active transporters that harness the transmembrane gradients of $[Na^+]$ and $[K^+]$ set by the ATP-dependent Na^+-K^+ pump as the driving force. NKCC1 imports Cl^- and thus increases the $[Cl^-]_i$, whereas KCC2 extrudes Cl^- from the cell, helping to maintain the $[Cl^-]_i$ low (Figure 1). The $[Cl^-]_i$ is maintained high in mammalian CNS neurones at early developmental stages such that the E_{GABA} is positive to the resting membrane potential (RMP) in these cells but, in later stages, the $[Cl^-]_i$ is usually reduced to low levels. In the rat, a postnatal increase in the expression and function of KCC2 is responsible for the reduction of $[Cl^-]_i$ and consequent shift of the E_{GABA} to a more negative potential.[12] Accordingly, the relative activities of NKCC1 and KCC2 are critical determinants of the E_{GABA}.

The polarity of membrane potential response to $GABA_A$ receptor activation depends on the relationship of the E_{GABA} to the membrane potential at the time the receptor is opened by GABA. In typical mature CNS neurones in which the $[Cl^-]_i$ is maintained low and hence the E_{GABA} is more negative than the RMP, $GABA_A$ receptor activation at the resting state makes the membrane potential move toward the E_{GABA}, producing a hyperpolarising response (Figure 1A). On the other hand, in cells with high $[Cl^-]_i$ and consequently having a depolarised E_{GABA} compared to the RMP, $GABA_A$ receptor stimulation leads to the depolarisation of membrane potential (Figure 1B). The magnitude of a given polarity of membrane potential response to $GABA_A$ receptor stimulation depends on the driving force (ie, the potential difference between E_{GABA} and the membrane potential at which $GABA_A$ receptor is activated): the larger the driving force and the $GABA_A$ conductance, the greater the magnitude of the membrane potential response.

The direct hyperpolarisation and depolarisation resulting from $GABA_A$ receptor activation may not necessarily correspond to inhibition and excitation of the neurone, respectively. The

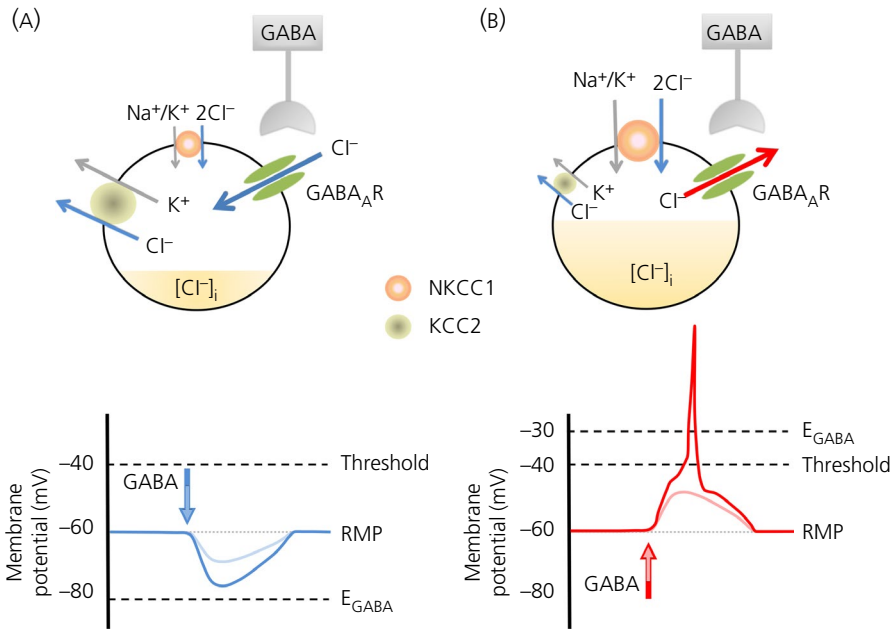

FIGURE 1 Schematic diagram showing $GABA_A$ signalling in neurones with different $[Cl^-]_i$. In central nervous system neurones, $[Cl^-]_i$ is usually determined by the relative activity of the Cl^- importer Na^+-K^+-$2Cl^-$ cotransporter 1 (NKCC1) vs the Cl^- extruder K^+-Cl^- cotransporter 2 (KCC2). In cells with relatively low $[Cl^-]_i$ and thus having relatively hyperpolarised E_{GABA} values (eg, −80 mV) as in (A), $GABA_A$ receptor activation causes the cell membrane potential to move towards the E_{GABA} by allowing chloride ions to flow into the cell through the open $GABA_A$ receptor. This membrane potential change generates a hyperpolarising response. The opposite pattern of events occur for cells with a relatively high $[Cl^-]_i$ and hence with relatively depolarised E_{GABA} values (eg, −30 mV) (B). In this case, the depolarising response from $GABA_A$ receptor activation can trigger action potential(s) if it is sufficiently large to reach the action potential threshold, which is a function of the rate of membrane potential change. It should be noted that HCO_3^- is not shown as a current carrier. $GABA_AR$, $GABA_A$ receptor; RMP, resting membrane potential

GABA-evoked hyperpolarisation can elicit rebound action potential bursts in some cells by removing the inactivation of (ie, deinactivating) certain voltage-gated channels, such as T-type Ca^{2+} channels.[13] Thus, the hyperpolarisation from $GABA_A$ receptor activation will not always result in inhibition of neural activity. Likewise, the depolarisation from $GABA_A$ receptor activation will not always result in an increase in neural activity. Although the depolarisation may evoke action potential by itself when it is sufficiently large to reach the threshold (Figure 1B) or via its spatiotemporal summation with other excitatory events such as excitatory postsynaptic potentials (EPSPs), it can also inhibit neuronal firing by so-called "shunting inhibition". Whenever the $GABA_A$ receptor channel is activated, the $GABA_A$ channel pore is open and hence the membrane conductance increases (ie, the membrane resistance decreases). Consequently, it becomes difficult for excitatory currents to alter the membrane potential to reach the action potential threshold.[14] Thus, prudence is required when classifying $GABA_A$ receptor-mediated responses as excitatory or inhibitory in regard to neural activity.

In the remainder of the review, we consider specific cases in hypothalamic cell populations in which $GABA_A$ signalling exhibits plasticity and can switch between evoking an inhibitory post-synaptic response and an excitatory one.

2.1 | Case 1: Circadian and seasonal plasticity of $GABA_A$ signalling in the suprachiasmatic nucleus (SCN) neurones

2.1.1 | Circadian time-dependent modulation of $GABA_A$ signalling in SCN neurones

It is now well-established that the SCN is the principal circadian clock responsible for generation of daily cycles of behaviour and physiology. SCN circuits underlie the generation of circadian

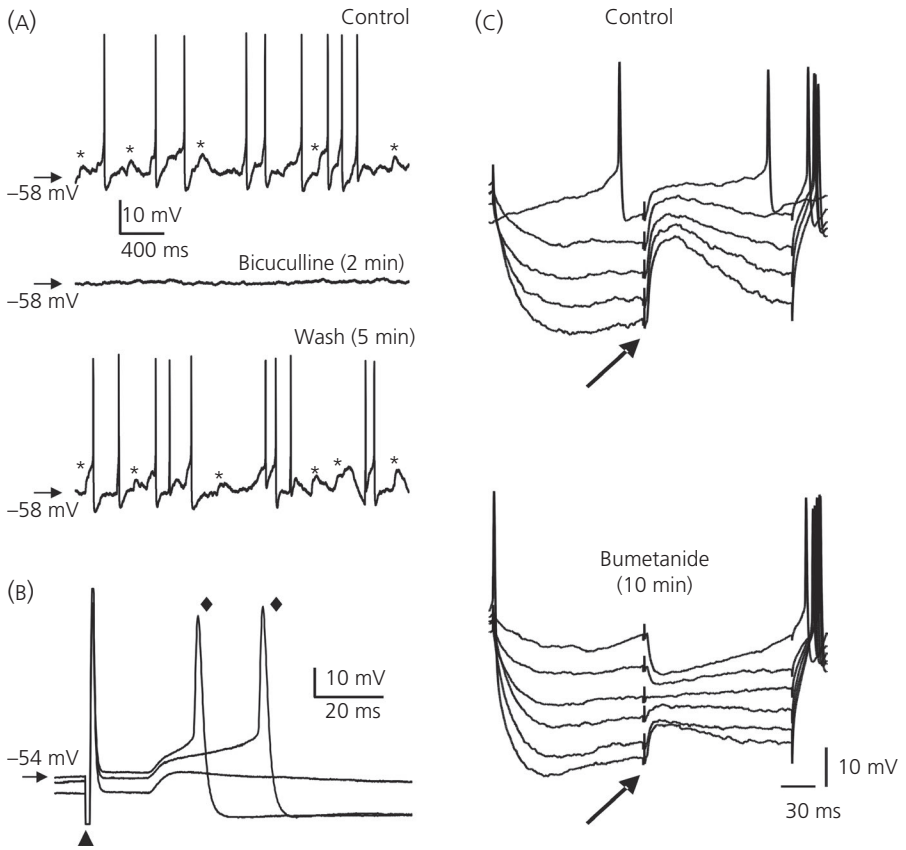

FIGURE 2 GABAergic excitation in suprachiasmatic nucleus (SCN) neurones. A, Reversible blockade of spontaneously occurring GABAergic excitatory postsynaptic potentials (EPSPs) by the $GABA_A$ receptor antagonist bicuculline (30 μmol L^{-1}). Voltage traces from an SCN neurone recorded in the presence of 6,7-dinitroquinoxaline-2,3-dione (DNQX; non-NMDA receptor antagonist) (20 μmol L^{-1}) and DL-2-amino-5-phosphonovalerate (DL-AP5; NMDA receptor antagonist) (100 μmol L^{-1}). B, Synaptic responses to optic nerve stimulation (results of three trials are shown) of an SCN neurone recorded before adding DNQX (20 μmol L^{-1}) and DL-AP5 (100 μmol L^{-1}) to the perfusion medium to isolate GABAergic EPSPs as shown in (A). This neurone responded to optic nerve stimulation (arrowhead) with glutamatergic EPSPs, which often triggered action potentials (diamonds). C, Effects of the Na^+-K^+-$2Cl^-$ cotransporter inhibitor bumetanide (10 μmol L^{-1}) on GABAergic EPSPs recorded in rat SCN neurones in the presence of DL-AP5 (100 μmol L^{-1}) and DNQX (20 μmol L^{-1}). The GABAergic PSPs were evoked at various membrane potentials by focal electrical stimulation (arrow) of the dorsolateral border of the SCN in the absence (top) and presence of bumetanide (bottom). The membrane potential was varied by injecting a series of current pulses (duration, 250 ms; intensity, 0 to −0.06 nA). Reproduced with permission from Choi et al[25]

rhythm and their environmental regulation, as well as the synchronisation of rhythmic outputs throughout the body.[15-18] A large body of evidence indicates that GABA, a neurotransmitter produced in a vast majority of SCN neurones and present in numerous synaptic terminals in the SCN, plays important roles in these processes.[19]

Although GABA commonly serves as an inhibitory transmitter in SCN neurones,[20-22] there has also been consistent evidence that GABA can be excitatory at certain times of the day and/or in certain subregions of the SCN.[23-29] For example, de Jeu and Pennartz[24] employed a gramicidin-perforated patch-clamp technique, which preserves the $[Cl^-]_i$ of the recorded cell,[30] and examined the effects of synaptically released or exogenously applied GABA in neurones in the SCN slices prepared from adult male Wistar rats held under a 12:12 hour light/dark (LD) cycle. They found that GABA was mostly inhibitory during the light phase, although it was excitatory during the dark phase in approximately 50% of the neurones sampled. Subsequently, Choi et al[25] employed extracellular single-unit recording and gramicidin-perforated patch-clamp techniques to survey a large number of neurones in the SCN slices, which were prepared from adult male Sprague-Dawley rats entrained to a 12:12 hour LD cycle. Although synaptically released or exogenously applied GABA inhibited most SCN neurones, GABAergic excitation was present in some neurones in both dorsal and ventral regions of the SCN, including a small proportion of retinorecipient cells (Figure 2A,B), regardless of the time of day, and these excitatory responses were most prevalent during the dark phase in the dorsal SCN. In individual neurones, the application of the NKCC blocker bumetanide converted GABAergic excitation to inhibition (Figure 2C), indicating that the Cl^--importing action of NKCC1 gives rise to the excitatory responses of SCN neurones to GABA. Ca^{2+}-imaging and western blot experiments also suggested that GABAergic excitation is dependent on NKCC1. It was shown that: (i) the GABA-elicited Ca^{2+} transient in acutely dissociated rat SCN neurones was inhibited reversibly by bumetanide; (ii) the GABA-elicited Ca^{2+} transients in acutely dissociated SCN neurones from NKCC1 null mice (30-40 days of age) were not inhibited by bumetanide, unlike those from their age-matched wild-type littermates; and (iii) NKCC1 protein content increased during the dark phase in the dorsal SCN of the adult mouse. Choi et al[25] showed that GABAergic excitation occurs more frequently during the dark phase in the dorsal SCN and is NKCC1-dependent, and their findings are consistent with the results of de Jeu and Pennartz[24] and have also been at least partially confirmed in subsequent studies.[27-29,31] However, the generality of the findings on the spatiotemporal pattern of GABAergic effects was questioned by Alamilla et al.[29] These investigators, on the basis of the results of gramicidin-perforated patch-clamp recordings in the SCN slices from adult male Wistar rats entrained to a 12:12 hour LD cycle, suggested that synaptically released GABA is excitatory and inhibitory in the dorsal and ventral SCN during the light phase, respectively, and vice versa in the dark phase. Collectively, the above work supports the proposition that, at least in certain SCN neurones, $GABA_A$ signalling is modulated in a time-dependent fashion such that GABA excites or inhibits these cells depending on the time of the day. Furthermore, these studies indicate that the increased NKCC1 activity underlies the emergence of the GABAergic excitation.

2.1.2 | Long-term ionic modulation in $GABA_A$ signalling in the SCN plays roles in circadian pacemaker resetting and seasonal time encoding

Despite the extensive body of data in the literatures indicating the long-term ionic modulation of $GABA_A$ signalling in the SCN, the functional significance of the GABAergic excitation is not yet fully resolved. The finding that GABA often excites some retinorecipient cells in the SCN during the dark (as well as light) phase[25,27] is consistent with the notion that the modulation of $GABA_A$ signalling may be important for the photic entrainment of the circadian pacemaker. McNeill et al[32] tested this hypothesis by examining the effects of the NKCC inhibitor bumetanide injected into the SCN in an attempt to block GABAergic excitation[25,27,32] on the photic (as well as non-photic) stimulus-induced phase shifts of the free-running circadian locomotor rhythms of male Syrian hamsters (9-10 weeks of age) housed under constant darkness (DD). It was found that prior injection of bumetanide into the SCN reduced the phase delays induced by light pulse presented to the animals in early subjective night [circadian time (CT): 13.5 hours], although not the phase advances induced by light in late subjective night (CT: 19 hours). Under DD conditions, circadian phase is defined relative to the onset of locomotor activity, with CT 12 hours being the phase at which locomotor activity starts in a nocturnal organism. Furthermore, it was discovered that bumetanide injection during the subjective day (CT 6 hours) attenuated the phase advances induced by co-injected muscimol ($GABA_A$ receptor agonist) into the SCN. Thus, evidence was provided results supporting the idea that GABAergic excitation plays a role in the environmental regulation of the circadian pacemaker.

In addition, long-term ionic modulation in $GABA_A$ signalling in the SCN may be important in encoding seasonal time. In support of this hypothesis, Farajnia et al[28] performed Ca^{2+} imaging experiments in SCN slices from mice (8-16 weeks of age) that were entrained to a long-day (18:8 hour LD cycle) or short-day (8:16 hour LD cycle) photoperiod. It was found that, compared with a short-day photoperiod, a long-day photoperiod was associated with significantly more excitatory and fewer inhibitory responses to GABA, and the excitatory responses were attenuated by bumetanide. The relationship between GABAergic excitation and inhibition may determine the phase distribution of individual clock cells in the SCN,[28] the change of which has been proposed as the mechanism encoding seasonal time.[33] In support of this hypothesis, Myung et al[34] measured circadian oscillations of the clock gene *Bmal1*, at single-cell and regional levels in cultured SCN explanted from mice raised under short or long days. They found that a phase gap between the oscillations recorded from the dorsal and ventral regions increases and also that the cycle-length of the SCN shortens when the mice were held on longer day lengths. In addition, an increasing day length alters the NKCC1/KCC2 ratio and increases resting intracellular chloride under

long days as imaged by N-(ethoxycarbonylmethyl)-6-methoxyquinolinium bromide fluorescence. Blocking $GABA_A$ signalling or the chloride transporter with furosemide (a non-selective NKCC1 and KCC2 inhibitor) prevented the long day-induced changes in phase and period. Mathematical modelling was used to support their interpretation that day length is encoded by the modulation of the intracellular chloride concentration, which can adjust the strength and polarity of the ionotropic $GABA_A$-mediated synaptic response. In sum, work carried out in the SCN provides clear evidence that GABA can function as an excitatory or inhibitory transmitter in this cell population. There is still controversy as to whether the excitatory/inhibitory effects of GABA occur more frequently in neurones at certain subregions of the SCN or during certain times of the day. In the cases in which GABAergic excitation has been clearly demonstrated, the mechanism depends on the NKCC1-dependent modulation of $[Cl^-]_i$ in SCN neurones. Although work on the functional significance is ongoing, recent studies indicate that long-term ionic modulation in $GABA_A$ signalling in the SCN plays crucial roles in photic entrainment of the circadian pacemaker and in encoding seasonal time. The mechanisms through which the molecular clock regulates E_{GABA} are not known. As described in this review, neuronal Cl^- homeostasis is regulated by the activity of two cation chloride co-transporters: the KCC2 and NKCC1. The activity of these transporters is determined mainly by their levels of expression in the plasma membrane. Furthermore, these chloride co-transporters are rapidly adjusted by activity-dependent post-translational modifications.[35, 36] There is good evidence that the transcript levels of the gene encoding NKCC1 (*Slc12a2*) are rhythmically expressed in tissues throughout the body, including liver, lung, heart and hypothalamus (http://circadb.hogeneschlab.org). In addition, in the SCN at least, there is compelling evidence for intracellular circadian rhythms in calcium, cAMP and other signalling pathways.[37] Accordingly, rhythms in E_{GABA} could be plausibly generated by either transcriptional or post-translational mechanisms. More work carried out in the future using targeted genetic manipulation of chloride transporters in SCN neurones will likely provide important insights.

2.2 | Case 2: Chronic hyperosmotic/hypernatraemic stress converts GABAergic inhibition to excitation in magnocellular neurosecretory cells (MNCs) to promote vasopressin and oxytocin release from these neurones

MNCs in the supraoptic (SON) and paraventricular nuclei (PVN) of the hypothalamus are critically involved in the regulation of osmotic balance. Vasopressin (VP) and oxytocin (OT) synthesised by these neurones are released either locally from their somata and dendrites[38,39] onto postsynaptic CNS neurones that they project to[40,41] or into the general circulation in the posterior pituitary.[42] VP released into the bloodstream increases water permeability in the collecting ducts of the nephron, promoting water retention (ie, antidiuresis).[43] Moreover, VP, at concentrations higher than those required for the V2 receptor-mediated antidiuretic action in the kidney, can cause vasoconstriction by stimulating the vascular smooth muscle via the V1a receptor.[44] These actions of VP are important with respect to maintaining the volume and osmolality of extracellular fluid (plasma) within physiological ranges, as well as raising blood pressure against hypotension resulting from a large reduction of blood volume, as occurs in severe haemorrhage or diarrhoea.[45] OT released into the systemic circulation plays an important role in reproduction in the female. OT facilitates birth by enhancing uterine contraction during parturition and induces milk ejection in the lactating mother by stimulating myoepithelial cells in the mammary gland lobules.[46] In some species (eg, rat), OT also exerts a natriuretic effect via its direct action in the kidney and by stimulating the release of atrial natriuretic hormone from the heart.[47]

Anatomical studies have shown that chronic dehydration and lactation leads to extensive and reversible remodelling in synapses and neurone-glia contact in the PVN and SON; both glutamatergic and GABAergic terminals making synaptic contacts with MNCs increase in number and the glial coverage of MNCs decreases significantly.[48-52] Electrophysiological studies have demonstrated that, corresponding to the anatomical changes, functional changes in glutamatergic and GABAergic inputs to MNCs also occur during dehydration and lactation.[53-56] For example, Di and Tasker[53] found that, in the rat, chronic dehydration led to a significant increase in the frequencies of spontaneously occurring glutamatergic and GABAergic postsynaptic currents in MNCs of the SON. More recently, a series of studies reported that chronic stimulation of MNCs promoting VP and/or OT release in various contexts was associated with changes in $GABA_A$ signalling in MNCs dependent on the modulation of $[Cl^-]_i$.[2,3,57,58]

To test the hypothesis that chronic hyperosmotic/hypernatraemic stress would weaken $GABA_A$ receptor-mediated inhibitory postsynaptic potentials (IPSPs) in MNCs to promote VP and OT release, Kim et al[2] salt-loaded adult male Sprague-Dawley rats by substituting drinking water with 2% NaCl solution for 7 days. Then, employing the gramicidin-perforated patch-clamp technique, they recorded $GABA_A$ receptor-mediated postsynaptic potentials (PSPs) in the MNCs sampled from acute hypothalamic slices obtained from these rats and from control animals kept on normal water. In these electrophysiological experiments, it was found that the chronic hyperosmotic/hypernatraemic stress given with the 2% saline drink not only attenuated GABAergic IPSPs in magnitude, but also converted them to EPSPs in a reversible fashion (Figure 3A). These EPSPs were blocked by the $GABA_A$ receptor antagonist bicuculline (Figure 3B) and mimicked by the $GABA_A$ receptor agonist muscimol (Figure 3C), indicating that they were $GABA_A$ receptor-mediated events. The conversion of GABAergic IPSPs to EPSPs was the result of a profound depolarising shift of E_{GABA} in MNCs, which was associated with increased expression of the Cl^- importer NKCC1 in MNCs and blocked by the NKCC inhibitor bumetanide, as well as by decreasing NKCC1 activity via a reduction of extracellular sodium. Remarkably, blocking central oxytocin receptors during the hyperosmotic/hypernatraemic stress prevented the switch to GABAergic excitation. Lastly,

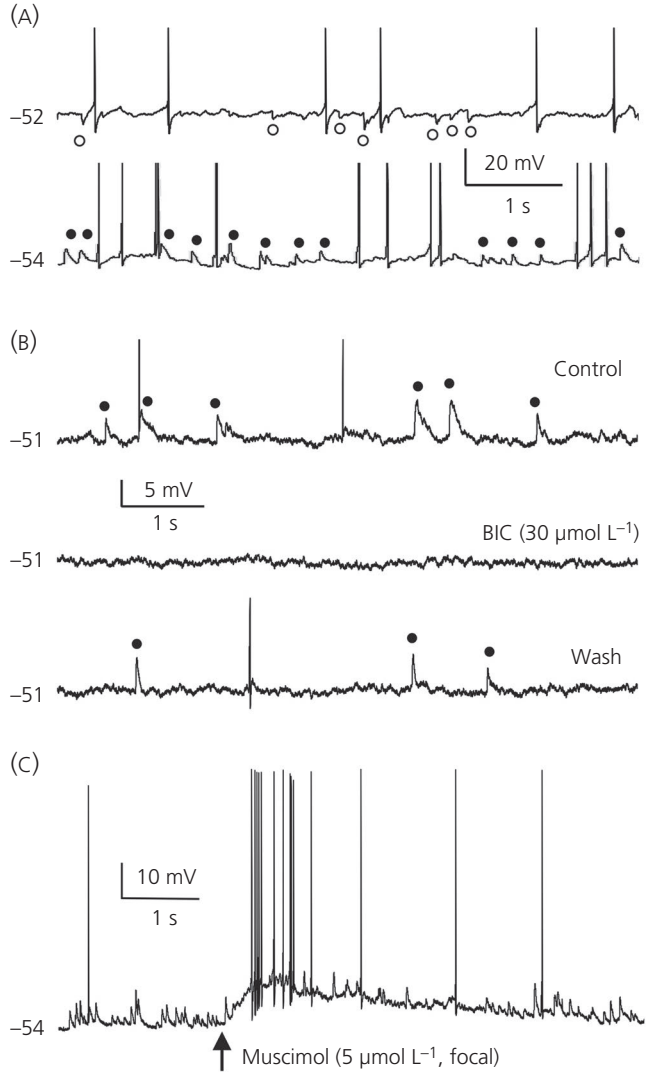

FIGURE 3 GABAergic inhibition and excitation in magnocellular neurosecretory cells (MNCs). A, Spontaneous GABAergic inhibitory postsynaptic potentials (IPSPs) (○, top trace) and excitatory postsynaptic potentials (EPSPs) (●, bottom trace) recorded in MNCs of the control rat and the experimental rat under chronic hyperosmotic stress, respectively. Resting membrane potential (RMP), −52 mV (cell with IPSPs), −54 mV (cell with EPSPs). B, Reversible blockade by bath-applied bicuculline (BIC) (30 μmol L^{-1}) of GABAergic EPSPs recorded in a cell from the rat under chronic hyperosmotic stress (RMP: −51 mV). Action potentials in (A) and (B) are truncated. C, Muscimol ($GABA_A$ receptor agonist, 5 μmol L^{-1}, 50 ms focal application)-induced depolarisation and spike generation in a cell from the rat under chronic hyperosmotic stress (RMP: −54 mV). All of the data shown were obtained after blocking glutamatergic transmission with DL-AP5 (100 μmol L^{-1}) and DNQX (20 μmol L^{-1}). Reproduced with permission from Kim et al[2]

i.v. injection of bicuculline at a subconvulsive dose lowered the plasma levels of VP and OT in rats under chronic stress but did not alter the VP and OT levels in control rats. These findings led to the conclusion that $GABA_A$ signalling in MNCs switches between inhibition and excitation in response to physiological needs and that the up-regulation of NKCC1 in MNCs, which is driven by the activation of central OT receptors, allows the inhibitory-to-excitatory switch to occur under chronically hyperosmotic/hypernatraemic conditions. The net result is that more VP and OT are released in response to this stressor.

The finding of Kim et al[2] showing that chronic hyperosmotic/hypernatraemic stress converts $GABA_A$ signalling in MNCs from inhibition to excitation was confirmed in subsequent studies performed with the deoxycorticosterone acetate (DOCA)-salt hypertension rat model and the streptozotocin (STZ)-induced diabetes mellitus (DM) rat model.[57,58] The DOCA-salt hypertension model, which partly depends on increased VP action,[59] was produced by injecting DOCA (25 mg) into the rat (adult male, Sprague-Dawley strain) once every 4 days for 4 weeks after unilateral nephrectomy and providing 1% NaCl solution as drinking water for this period, whereas the STZ-induced DM model was produced by injecting the antineoplastic drug STZ (65 mg/kg body weight, i.p. single injection) into the rat (adult male, Sprague-Dawley or Wistar strain). Streptozotocin is known to kill insulin-producing pancreatic β-cells, and the DM induced by this agent is characterised by high levels of plasma VP, contributing to the genesis of DM-associated nephropathy either directly or by aggravating hyperglycaemia.[60] The plasma osmolality was higher in these rat models than in vehicle-treated intact control animals as a result of hypernatraemia or hyperglycaemia. Also, as expected, the plasma VP levels were higher (5-12 times) in the rat models. Kim et al[57,58] found that $GABA_A$ receptor-mediated IPSPs in VP-secreting MNCs were converted to EPSPs in the rat models as a result of the depolarising shift of E_{GABA}, thus obtaining evidence that the inhibitory-to-excitatory switch of $GABA_A$ signalling is a key mechanism underlying the high plasma levels of VP in the STZ-induced DM and DOCA-salt hypertension models. Moreover, it was discovered that the shift of E_{GABA} was a result of NKCC1 up-regulation and KCC2 down-regulation in MNCs. Evidence for these mechanisms was provided via western blot experiments performed with SON and/or PVN tissues and neurophysiological experiments evaluating the effects of the NKCC inhibitor bumetanide, as well as the KCC2 inhibitors VU0240551 (known to be less specific) or VU0463271 (known to be specific and effective), on E_{GABA} and the GABAergic response profile (ie, the ratio of cells showing GABAergic EPSPs vs IPSPs) of the VP-secreting MNCs of control and model rats. The evidence for KCC2 down-regulation in the STZ model was further provided by experiments performed using the KCC2 activator CLP257 and its prodrug CLP290, which are drugs that activate KCC2 by apparent inhibition of the action of brain-derived neurotrophic factor (BDNF).[61] Kim et al[58] demonstrated that CLP257 hyperpolarised the E_{GABA} and hence converted GABAergic excitation back to inhibition in the VP-secreting MNCs of STZ model rats. Moreover, they showed that i.p. injection of CLP 290 significantly lowered the VP and glucose levels in the blood in STZ-treated but not control, rats. These findings not only support the notion that the down-regulation of KCC2 is partly responsible for the depolarising shift of E_{GABA}, but also suggest that CLP290 is an effective means of lowering the high blood levels of VP and glucose in DM, comprising characteristic features of this metabolic disorder that may contribute to its progression[62,63] and directly drive serious DM complications such as renal failure.[60]

Choe et al[64] further confirmed and extended the finding of Kim et al[2,57] showing that the inhibitory-to-excitatory switch of $GABA_A$ signalling occurs in MNCs under chronic hyperosmotic/hypernatraemic stress. These investigators showed that the conversion of GABAergic inhibition to excitation in MNCs occurred in all different strains of the salt-loaded (substitution of drinking water with 2% saline for 7 days) rats examined so far (Wistar, Sprague-Dawley, Long-Evans, Fischer 344), indicating that the plasticity of $GABA_A$ signalling is not rat strain-specific. More importantly, with Long-Evans rats, it was also demonstrated that the BDNF-dependent activation of tropomyosin-receptor-kinase B (TrkB) underpins the down-regulation of KCC2, which is the molecular change identified as being responsible for the depolarising shift of E_{GABA} and the resultant conversion of GABAergic inhibition to excitation in MNCs. Specifically, they showed that: (i) furosemide had no effect on the E_{GABA} in the MNCs of salt-loaded rats, whereas it depolarised the E_{GABA} in euhydrated control rats; (ii) the level of KCC2 expression in the SON was lower in salt-loaded than in control rats; (iii) the salt-loading increased the phosphorylated TrkB (pTrkB) level in the SON, whereas scavenging TrkB agonist molecules in the SON with TrkB-Fc during the salt-loading prevented the depolarising shift of E_{GABA} from occurring in SON MNCs; and (iv) the knockdown of BDNF in the SON with short-hairpin RNA directed against BDNF prevented the salt-loading from depolarising the E_{GABA} of SON MNCs.

Choe et al[64] excluded the role for NKCC1 in the depolarising shift of E_{GABA} induced by chronic hyperosmotic/hypernatraemic stimulation, in contrast to the conclusions of Kim et al[2,57,58] These investigators obtained evidence for the role of NKCC1 via in vitro studies involving western blot, immunohistochemistry and/or brain slice-based neurophysiology experiments.[2,57,58] In addition, they demonstrated that bumetanide (NKCC inhibitor) infused i.c.v. for a week between days 14 and 21 of DOCA-salt treatment of the rat lowered the plasma VP level,[57] although the central infusion of bumetanide might have had non-specific effects. It is unclear why Choe et al[64] could not obtain evidence for the role of NKCC1. One possibility is that, in Long-Evans rats (ie, strain of rats these investigators used), the regulation of $[Cl^-]_i$ in MNCs depends not so much on NKCC1 but, instead, on other Cl^- transporter(s) such as KCC2, as discussed by Choe et al.[64]

2.2.1 | Chronic hyperosmotic/hypernatraemic stress alters baroreflex inhibition of MNCs

The findings of Kim et al[2,57,58] and Choe et al[64] showing that the E_{GABA} is depolarised in the MNCs of salt-loaded rats and DOCA-salt and STZ model rats predict that the well-known baroreflex inhibition of MNCs mediated by $GABA_A$ signalling is reduced or even converted to excitation in these animals. Kim et al[57] tested this possibility in urethane-anaesthetised DOCA-salt model rats (Sprague-Dawley, male). They examined the effects of raising the blood pressure with an i.v. injection of the α-adrenergic agonist phenylephrine on the extracellularly recorded single-unit activities of MNCs in the SON, which were antidromically identified as projecting to the posterior pituitary, as well as on the VP levels in the plasma. In these experiments, Kim et al[57] found that, in control rats, baroreceptor activation by phenylephrine injection mostly resulted in the inhibition of the MNCs (11 of 14 neurones) whereas, in the DOCA-salt model rats, it mostly excited MNCs (12 of 16 neurones). Also, they discovered that phenylephrine injection led to a significant rise in the plasma VP concentration in the model rats but had no effect on the plasma VP level in control rats. Taken together, the results of Kim et al[57] strongly indicate that chronic hyperosmotic/hypernatraemic stress converts the baroreflex inhibition of VP-secreting MNCs to excitation, such that an increase in blood pressure paradoxically promotes the output of these neurones. This conclusion is supported by recent studies carried out in other laboratories with different strains of rats. For example, in an in vivo electrophysiological study performed with male Long-Evans rats, Choe et al[64] obtained results similar to those of Kim et al.[57] That is, baroreceptor activation in salt-loaded rats excited a significant proportion of VP-secreting MNCs (six of 17 cells) whereas, in euhydrated control rats, it inhibited most VP-secreting MNCs (nine of 10 cells). Han et al[65] and Korpal et al[66] demonstrated that the baroreflex inhibition of VP-secreting MNCs was blunted in the angiotensin II-dependent hypertension model rats (Fischer strain, male). This model of hypertension, driven by over-activation of the renin-angiotensin-aldosterone system (RAAS), is produced by administering indole-3-carbinol to the rat having Cyp1a1-Ren2 transgene in Y-chromosome.[67]

The functional significance of an altered baroreflex in VP-secreting MNCs is unclear. The increase in VP release that results from the altered baroreflex may be quite substantial, and sufficient to cause hypertension. Consistent with this hypothesis, Choe et al[64] and Korpal et al[66] found that systemic administration of V1a VP receptor antagonist suppressed the blood pressure increases induced by salt-loading and RAAS over-activation, respectively. Moreover, Kim et al[57] discovered that i.c.v. administration of bumetanide during DOCA-salt treatment lowered the plasma VP level (see above) and delayed the development of hypertension, whereas local injection of muscimol ($GABA_A$ receptor agonist) into the SON increased blood pressure in a manner that could be blocked by i.v. injection of V1a VP receptor antagonist in DOCA-salt model rats. It is still not clear whether the altered baroreflex is causally linked to the increased VP release. The increase in VP release might be a result not only of the altered baroreflex in MNCs, but also other factors, such as the changes of $GABA_A$ receptor-mediated events other than baroreflex and increased glutamatergic transmission,[53] and these factors might be the major contributors to the increased VP release. Furthermore, it is possible that increased VP release under certain chronic hyperosmotic/hypernatraemic conditions or in certain rat strains does not contribute to the generation of hypertension. Indeed, Balapattabi et al[68] showed that, in salt-loaded Sprague-Dawley rats, the knockdown of BDNF in the SON with short-hairpin RNA directed against BDNF had no effect on the rise of blood pressure induced by salt loading, despite it preventing salt-loading from increasing VP release from the pituitary. More work is required to determine whether the

increase in VP release as a result of the altered baroreflex contributes significantly to the generation of hypertension.

2.3 | Case 3: Lactation weakens GABAergic inhibition or converts it to excitation in MNCs

Lactation is associated with the increased outputs of VP- and OT-secreting MNCs, which ensure the delivery of sufficient milk to the offspring and prevent maternal dehydration.[69,70] The increased release of VP and OT may partly be a result of the reduction of inhibitory $GABA_A$ signalling in MNCs. As a step toward testing this hypothesis, Lee et al[3] examined whether $GABA_A$ signalling in MNCs in female rats is modulated in a reproductive stage-dependent fashion. They found that lactation (just as chronic hyperosmotic/hypernatraemic stress) caused the E_{GABA} to depolarise in MNCs, hence suppressing GABAergic inhibition or even converting it to excitation in these neurones. Specifically, the investigators discovered that: (i) the E_{GABA} was significantly depolarised in MNCs in the lactating rats compared to virgins; (ii) the depolarising shift in E_{GABA} in MNCs was much larger in magnitude in more experienced mothers such that GABA exerted an excitatory, instead of inhibitory, effect in most of the MNCs (both VP- and OT-secreting) of multiparous rats; and (iii) the E_{GABA} was less negative in rats in dry period after three reproductive cycles than in those in first lactation. Neurophysiological results indicated that the up-regulation of NKCC1 and down-regulation of KCC2 give rise to the depolarising shift of E_{GABA} in the MNCs. Collectively, these findings suggest that lactation depolarises the E_{GABA} in MNCs to weaken GABAergic inhibition or to convert it to excitation in these neurones. In addition, the findings of Lee et al[3] suggest that, in the MNCs of multiparous rats in lactation, the E_{GABA} is profoundly depolarised partly because the depolarising E_{GABA} shift induced by previous lactation is not fully reversed during the next dry period. Still, more work is required to establish whether these lactation-associated changes in $GABA_A$ signalling in VP- and OT-secreting MNCs represent one of the mechanisms underlying the increased hormonal secretion of these cells during lactation.

2.4 | Case 4: Up-regulation of NKCC1 blunts GABAergic inhibition in presympathetic parvocellular neurones in the PVN to increase sympathetic outflow in spontaneously hypertensive rats (SHRs)

Parvocellular PVN neurones projecting to the sympathetic-related regions in the brainstem and spinal cord play an important role in regulating sympathetic outflow.[71-74] The activities of these presympathetic PVN neurones are finely tuned by excitatory and inhibitory synaptic inputs.[75] Hence, the imbalance of excitation/inhibition in presympathetic neurones may be the basis of elevated sympathetic outflow in hypertension.[76-81] For example, studies performed with SHRs have found that reduced inhibitory GABAergic (in addition to enhanced excitatory glutamatergic) inputs to presympathetic neurones underlie the increased sympathetic outflow in this hypertension model rats.[75] In particular, a study by Ye et al[82] has provided data suggesting that the impairment of GABAergic inhibition resulting from the modulation of $[Cl^-]_i$ in presympathetic neurones is crucial for the increase of sympathetic outflow. These investigators found that, as a result of a significant depolarising shift of E_{GABA}, GABAergic inhibition was blunted in the presympathetic neurones of SHRs, and also that incubating the hypothalamic slices from SHRs with bumetanide completely normalised the E_{GABA} and hence restored GABAergic inhibition in these cells. These findings suggest that NKCC1 up-regulation underlies the E_{GABA} shift. Ye et al[82] further discovered that the level of glycosylated (but not non-glycosylated) NKCC1 protein was significantly higher in the PVN of SHRs than in normotensive Wistar-Kyoto (WKY) rats, although the KCC2 level in the PVN was not different between SHRs and WKY rats. Incubating the hypothalamic slices from SHRs with the N-linked glycosylation inhibitor tunicamycin also normalised the E_{GABA} and restored GABAergic inhibition in presympathetic neurones. The central administration of bumetanide in SHRs decreased sympathetic outflow and arterial blood pressure and made muscimol ($GABA_A$ receptor agonist) injected into the PVN evoke greater sympathoinhibitory responses. Thus, the findings of Ye et al[82] indicate that increased glycosylated NKCC1 blunts GABAergic inhibition in presympathetic neurones to induce and/or maintain hypertension in SHRs by elevating sympathetic outflow. Whether or not the change in $GABA_A$ signalling in presympathetic neurones is a general feature in hypertension remains to be determined.

2.5 | Case 5: Stress alters Cl^- homeostasis in corticotrophin-releasing hormone (CRH) neurones to remove inhibitory GABAergic constraint of the hypothalamic-pituitary-adrenal (HPA) axis

Parvocellular CRH neurones in the PVN respond to stress with the release of CRH, which in turn increases the blood glucocorticoid levels via corticotrophin secretion from the anterior pituitary.[83,84] The neural activity and hormonal output of CRH neurones are tightly regulated by inhibitory GABAergic inputs,[1,85] arising from interneurones distributed near the PVN.[1,85,86] As such, it has been proposed that the release of CRH neurones from GABAergic inhibition is essential for the initiation of neuroendocrine response to stress.[87,88] Using an acute restraint stress protocol combined with in vivo microinjections, hormone measurements and patch-clamp recordings from hypothalamic slices prepared from control and stressed animals, Hewitt et al[89] examined the mechanisms underlying the release of the GABAergic inhibition. They found that: (i) blockade of $GABA_A$ signalling in PVN with bicuculline microinjection had no effect on the circulating level of corticostrone (CORT) in stressed animals, whereas, in control rats, it elicited a robust increase in the blood level of the corticosteroid; (ii) E_{GABA} was depolarised in the putative CRH neurones of stressed rats, and bath-applied furosemide (200 μmol L^{-1}) depolarised the E_{GABA} of putative CRH neurones recorded in the hypothalamic slices from unstressed control rats, although it had no effect in stressed rats; (iii) unlike furosemide, bumetanide (10 μmol L^{-1}) had no effect on E_{GABA} in the cells of control

rats; (iv) in control rats, furosemide microinjection into the PVN elicited a robust increase in circulating CORT; (v) the expression levels of KCC2 protein in the PVN were not different between the control and stressed rats; (vi) the depression of inhibitory GABAergic responses of putative CRH neurones occurring during high-frequency stimulation was more pronounced in the stressed than control rats; and (vii) after stress, repetitive stimulation of GABAergic input under specific conditions could transiently elicit GABAergic excitation in putative CRH neurones. Based on these results, Hewitt et al[89] concluded that acute stress decreases the Cl^--extruding capacity of KCC2 in putative CRH neurones to depolarise E_{GABA} in these cells and hence removes the inhibitory GABAergic constraint on the HPA axis. In their study, however, the possibility that increased NKCC1 activity contributes to the depolarising shift of E_{GABA} was not ruled out. Critically, the effect of blocking NKCC1 activity (eg, with bumetanide) on E_{GABA} in the putative CRH neurones of stressed rats was not examined. The lack of bumetanide effect demonstrated in the cells of control rats was expected because NKCC1 expression is normally low in the PVN in adult rat.[90] Furthermore, the effects of NKCC antagonist (eg, bumetanide) injected locally into the PVN on the circulating levels of CORT in both control and stressed rats were not examined. Finally, we note that furosemide is not selective and blocks both NKCC1 and KCC2. Therefore, the relative roles of NKCC1 and KCC2 in mediating the depolarising E_{GABA} shift remain to be clarified.

2.5.1 | Chronic unpredictable mild stress induces hyperactivity in the HPA by removing the GABAergic inhibition of CRH neurones through NKCC1 up-regulation

A more recent study[91] examined the effects of chronic unpredictable mild stress (CUMS) on the inhibitory $GABA_A$ signalling in CRH neurones. CUMS was inflicted by exposing the rat to two randomly chosen stressors per day from a total of eight stressors (cage rotation, cold isolation, light off, light on, forced swim, restraint stress, isolation housing and food/water deprivation) for 11 days. In unstressed rats, microinjection of gabazine ($GABA_A$ receptor antagonist) into the PVN resulted in a significant increase in plasma CORT levels, whereas, in CUMS rats, it had no significant effect on the CORT levels. Interestingly, CUMS caused a depolarising shift in E_{GABA} in CRH neurones, making GABA excitatory in these cells. The E_{GABA} shift was associated with a long-lasting increase in NKCC1 protein level and an early transient decrease in KCC2 protein level in the PVN and was normalised by bumetanide treatment. Moreover, in CUMS rats, i.c.v. administration of bumetanide resulted in a significant decrease in CORT levels, whereas it did not alter circulating CORT levels in unstressed control rats. Taken together, these results reported by Gao et al[91] indicate that chronic stress impairs GABAergic inhibition in CRH neurones via the up-regulation of NKCC1. In addition, the results raise the possibility that KCC2 down-regulation also contributes to the impairment of GABAergic inhibition in the early phase of post CUMS.

3 | PERSPECTIVES

In this review, we have presented evidence indicating that, in response to physiological needs or in association with pathological states such as hypertension, postsynaptic $GABA_A$ signalling can switch from functionally inhibitory to excitatory. The mechanism is dependent upon the modulation of postsynaptic $[Cl^-]_i$ as a result of the up-regulation of NKCC1 and/or the down-regulation of KCC2. There is a very extensive and rapidly growing literature on the role of (de)phosphorylation and intracellular signalling mechanisms targeting individual amino acids to modulate the activity of both NKCC1 and KCC2.[4] Moreover, these mechanisms appear to be universal, regardless of neuronal types. Future studies may use this information on NKCC1 and KCC2 modulation (typically involving changes at the level of membrane insertion of the transporters) to aid in the design of experiments on neuroendocrine cells, and perhaps also for novel therapies.

ACKNOWLEDGEMENTS

This work was supported by the National Research Foundation of Korea (NRF) grants funded by the Korea government (MSIP) to Y. I. Kim (2017R1A2B2002277) and Y.-B. Kim (2019R1I1A1A01057744) and by the Korea University grant to Y.-B. Kim.

ORCID

Yang In Kim https://orcid.org/0000-0003-3420-7870

REFERENCES

1. Decavel C, Van den Pol AN. GABA: a dominant neurotransmitter in the hypothalamus. *J Comp Neurol*. 1990;302:1019-1037.
2. Kim JS, Kim WB, Kim YB, et al. Chronic hyperosmotic stress converts gabaergic inhibition into excitation in vasopressin and oxytocin neurons in the rat. *J Neurosci*. 2011;31:13312-13322.
3. Lee SW, Kim YB, Kim JS, et al. Gabaergic inhibition is weakened or converted into excitation in the oxytocin and vasopressin neurons of the lactating rat. *Mol Brain*. 2015;8:34.
4. Kaila K, Price TJ, Payne JA, Puskarjov M, Voipio J. Cation-chloride cotransporters in neuronal development, plasticity and disease. *Nat Rev Neurosci*. 2014;15:637-654.
5. Herbison AE, Moenter SM. Depolarising and hyperpolarising actions of GABA(a) receptor activation on gonadotrophin-releasing hormone neurones: towards an emerging consensus. *J Neuroendocrinol*. 2011;23:557-569.
6. Kaila K, Voipio J. Postsynaptic fall in intracellular ph induced by GABA-activated bicarbonate conductance. *Nature*. 1987;330:163-165.
7. Kaila K, Pasternack M, Saarikoski J, Voipio J. Influence of GABA-gated bicarbonate conductance on potential, current and intracellular chloride in crayfish muscle fibres. *J Physiol*. 1989;416:161-181.
8. Kaila K. Ionic basis of gabaa receptor channel function in the nervous system. *Prog Neurobiol*. 1994;42:489-537.
9. Fatima-Shad K, Barry PH. Anion permeation in GABA- and glycine-gated channels of mammalian cultured hippocampal neurons. *Proc Biol Sci*. 1993;253:69-75.

10. Ben-Ari Y. Excitatory actions of GABA during development: the nature of the nurture. *Nat Rev Neurosci*. 2002;3:728-739.
11. Blaesse P, Airaksinen MS, Rivera C, Kaila K. Cation-chloride cotransporters and neuronal function. *Neuron*. 2009;61:820-838.
12. Rivera C, Voipio J, Payne JA, et al. The k+/cl- co-transporter kcc2 renders GABA hyperpolarizing during neuronal maturation. *Nature*. 1999;397:251-255.
13. Suzuki S, Rogawski MA. T-type calcium channels mediate the transition between tonic and phasic firing in thalamic neurons. *Proc Natl Acad Sci USA*. 1989;86:7228-7232.
14. Staley KJ, Mody I. Shunting of excitatory input to dentate gyrus granule cells by a depolarizing gabaa receptor-mediated postsynaptic conductance. *J Neurophysiol*. 1992;68:197-212.
15. Meijer JH, Michel S. Neurophysiological analysis of the suprachiasmatic nucleus: a challenge at multiple levels. *Methods Enzymol*. 2015;552:75-102.
16. Pauls SD, Honma KI, Honma S, Silver R. Deconstructing circadian rhythmicity with models and manipulations. *Trends Neurosci*. 2016;39:405-419.
17. Allen CN, Nitabach MN, Colwell CS. Membrane currents, gene expression, and circadian clocks. *Cold Spring Harb Perspect Biol*. 2017;9:pii: a027714.
18. Hastings MH, Maywood ES, Brancaccio M. Generation of circadian rhythms in the suprachiasmatic nucleus. *Nat Rev Neurosci*. 2018;19:453-469.
19. Albers HE, Walton JC, Gamble KL, McNeill JK, Hummer DL. The dynamics of GABA signaling: revelations from the circadian pacemaker in the suprachiasmatic nucleus. *Front Neuroendocrinol*. 2017;44:35-82.
20. Gribkoff VK, Pieschl RL, Wisialowski TA, et al. A reexamination of the role of GABA in the mammalian suprachiasmatic nucleus. *J Biol Rhythms*. 1999;14:126-130.
21. Gribkoff VK, Pieschl RL, Dudek FE. GABA receptor-mediated inhibition of neuronal activity in rat scn in vitro: pharmacology and influence of circadian phase. *J Neurophysiol*. 2003;90:1438-1448.
22. Kononenko NI, Dudek FE. Mechanism of irregular firing of suprachiasmatic nucleus neurons in rat hypothalamic slices. *J Neurophysiol*. 2004;91:267-273.
23. Wagner S, Castel M, Gainer H, Yarom Y. GABA in the mammalian suprachiasmatic nucleus and its role in diurnal rhythmicity. *Nature*. 1997;387:598-603.
24. De Jeu M, Pennartz C. Circadian modulation of GABA function in the rat suprachiasmatic nucleus: excitatory effects during the night phase. *J Neurophysiol*. 2002;87:834-844.
25. Choi HJ, Lee CJ, Schroeder A, et al. Excitatory actions of GABA in the suprachiasmatic nucleus. *J Neurosci*. 2008;28:5450-5459.
26. Albus H, Vansteensel MJ, Michel S, Block GD, Meijer JH. A gabaergic mechanism is necessary for coupling dissociable ventral and dorsal regional oscillators within the circadian clock. *Curr Biol*. 2005;15:886-893.
27. Irwin RP, Allen CN. Gabaergic signaling induces divergent neuronal ca2+responses in the suprachiasmatic nucleus network. *Eur J Neurosci*. 2009;30:1462-1475.
28. Farajnia S, van Westering TLE, Meijer JH, Michel S. Seasonal induction of gabaergic excitation in the central mammalian clock. *Proc Natl Acad Sci USA*. 2014;111:9627-9632.
29. Alamilla J, Perez-Burgos A, Quinto D, Aguilar-Roblero R. Circadian modulation of the Cl- equilibrium potential in the rat suprachiasmatic nuclei. *Biomed Res Int*. 2014;2014: https://doi.org/10.1155/2014/424982.
30. Rhee JS, Ebihara S, Akaike N. Gramicidin perforated patch-clamp technique reveals glycine-gated outward chloride current in dissociated nucleus solitarii neurons of the rat. *J Neurophysiol*. 1994;72:1103-1108.
31. Belenky MA, Sollars PJ, Mount DB, et al. Cell-type specific distribution of chloride transporters in the rat suprachiasmatic nucleus. *Neuroscience*. 2010;165:1519-1537.
32. McNeill JK, Walton JC, Albers HE. Functional significance of the excitatory effects of GABA in the suprachiasmatic nucleus. *J Biol Rhythms* 2018;33:376-387.
33. VanderLeest HT, Houben T, Michel S, et al. Seasonal encoding by the circadian pacemaker of the scn. *Curr Biol*. 2007;17:468-473.
34. Myung J, Hong S, DeWoskin D, et al. GABA-mediated repulsive coupling between circadian clock neurons in the scn encodes seasonal time. *Proc Natl Acad Sci USA*. 2015;112:E3920-E3929.
35. Kahle KT, Delpire E. Kinase-kcc2 coupling: Cl^- rheostasis, disease susceptibility, therapeutic target. *J Neurophysiol*. 2016;115:8-18.
36. Come E, Marques X, Poncer JC, Levi S. Kcc2 membrane diffusion tunes neuronal chloride homeostasis. *Neuropharmacology*. 2019; https://doi.org/10.1016/j.neuropharm.2019.03.014. [Epub ahead of print].
37. Hastings MH, Maywood ES, Brancaccio M. The mammalian circadian timing system and the suprachiasmatic nucleus as its pacemaker. *Biology (Basel)*. 2019;8:pii: E13.
38. Ludwig M. Dendritic release of vasopressin and oxytocin. *J Neuroendocrinol*. 1998;10:881-895.
39. Pow DV, Morris JF. Dendrites of hypothalamic magnocellular neurons release neurohypophysial peptides by exocytosis. *Neuroscience*. 1989;32:435-439.
40. Knobloch HS, Charlet A, Hoffmann LC, et al. Evoked axonal oxytocin release in the central amygdala attenuates fear response. *Neuron*. 2012;73:553-566.
41. Knobloch HS, Grinevich V. Evolution of oxytocin pathways in the brain of vertebrates. *Front Behav Neurosci*. 2014;8:31.
42. Bicknell RJ. Optimizing release from peptide hormone secretory nerve terminals. *J Exp Biol*. 1988;139:51-65.
43. Robertson GL, Shelton RL, Athar S. The osmoregulation of vasopressin. *Kidney Int*. 1976;10:25-37.
44. Henderson KK, Byron KL. Vasopressin-induced vasoconstriction: two concentration-dependent signaling pathways. *J Appl Physiol*. 2007;102:1402-1409.
45. Rocha ESM Jr, Rosenberg M. The release of vasopressin in response to haemorrhage and its role in the mechanism of blood pressure regulation. *J Physiol*. 1969;202:535-557.
46. Leng G, Pineda R, Sabatier N, Ludwig M. 60 years of neuroendocrinology the posterior pituitary, from geoffrey harris to our present understanding. *J Endocrinol*. 2015;226:T173-T185.
47. Haanwinckel MA, Elias LK, Favaretto AL, et al. Oxytocin mediates atrial natriuretic peptide release and natriuresis after volume expansion in the rat. *Proc Natl Acad Sci USA*. 1995;92:7902-7906.
48. Hatton GI. Function-related plasticity in hypothalamus. *Annu Rev Neurosci*. 1997;20:375-397.
49. Miyata S, Nakashima T, Kiyohara T. Structural dynamics of neural plasticity in the supraoptic nucleus of the rat hypothalamus during dehydration and rehydration. *Brain Res Bull*. 1994;34:169-175.
50. Theodosis DT, Poulain DA. Activity-dependent neuronal-glial and synaptic plasticity in the adult mammalian hypothalamus. *Neuroscience*. 1993;57:501-535.
51. Chapman DB, Theodosis DT, Montagnese C, Poulain DA, Morris JF. Osmotic stimulation causes structural plasticity of neurone-glia relationships of the oxytocin but not vasopressin secreting neurones in the hypothalamic supraoptic nucleus. *Neuroscience*. 1986;17:679-686.
52. Tasker JGBC, Poulain DA, Theodosis DT. Cell biology of oxytocin and vasopressin cells. In: Pfaff DW, Arnold AP, Etgen AM, Fahrback SE, Rubin RT, eds. *Hormones, brain and behavior*, vol. 3. New York, NY: Academic Press; 2002:811-842.
53. Di S, Tasker JG. Dehydration-induced synaptic plasticity in magnocellular neurons of the hypothalamic supraoptic nucleus. *Endocrinology*. 2004;145:5141-5149.

54. Brussaard AB, Kits KS. Changes in gabaa receptor-mediated synaptic transmission in oxytocin neurons during female reproduction: plasticity in a neuroendocrine context. *Ann NY Acad Sci*. 1999;868:677-680.
55. Oliet SH, Piet R, Poulain DA. Control of glutamate clearance and synaptic efficacy by glial coverage of neurons. *Science*. 2001;292:923-926.
56. Stern JE, Hestrin S, Armstrong WE. Enhanced neurotransmitter release at glutamatergic synapses on oxytocin neurones during lactation in the rat. *J Physiol*. 2000;526:109-114.
57. Kim YB, Kim YS, Kim WB, et al. Gabaergic excitation of vasopressin neurons: possible mechanism underlying sodium-dependent hypertension. *Circ Res*. 2013;113:1296-1307.
58. Kim YB, Kim WB, Jung WW, et al. Excitatory gabaergic action and increased vasopressin synthesis in hypothalamic magnocellular neurosecretory cells underlie the high plasma level of vasopressin in diabetic rats. *Diabetes*. 2018;67:486-495.
59. Berecek KH, Murray RD, Gross F, Brody MJ. Vasopressin and vascular reactivity in the development of doca hypertension in rats with hereditary diabetes insipidus. *Hypertension*. 1982;4:3-12.
60. Bardoux P, Martin H, Ahloulay M, et al. Vasopressin contributes to hyperfiltration, albuminuria, and renal hypertrophy in diabetes mellitus: study in vasopressin-deficient brattleboro rats. *Proc Natl Acad Sci USA*. 1999;96:10397-10402.
61. Gagnon M, Bergeron MJ, Lavertu G, et al. Chloride extrusion enhancers as novel therapeutics for neurological diseases. *Nat Med*. 2013;19:1524–1528.
62. Yibchok-anun S, Abu-Basha EA, Yao CY, Panichkriangkrai W, Hsu WH. The role of arginine vasopressin in diabetes-associated increase in glucagon secretion. *Regul Pept*. 2004;122:157-162.
63. Bodnar RJ, Wallace MM, Kordower JH, et al. Modulation of nociceptive thresholds by vasopressin in the brattleboro and normal rat. *Ann NY Acad Sci*. 1982;394:735-739.
64. Choe KY, Han SY, Gaub P, et al. High salt intake increases blood pressure via bdnf-mediated downregulation of kcc2 and impaired baroreflex inhibition of vasopressin neurons. *Neuron*. 2015;85:549-560.
65. Han SY, Bouwer GT, Seymour AJ, et al. Induction of hypertension blunts baroreflex inhibition of vasopressin neurons in the rat. *Eur J Neurosci*. 2015;42:2690-2698.
66. Korpal AK, Han SY, Schwenke DO, Brown CH. A switch from GABA inhibition to excitation of vasopressin neurons exacerbates the development angiotensin ii-dependent hypertension. *J Neuroendocrinol*. 2017; Dec 9. https://doi.org/10.1111/jne.12564. [Epub ahead of print].
67. Kantachuvesiri S, Fleming S, Peters J, et al. Controlled hypertension, a transgenic toggle switch reveals differential mechanisms underlying vascular disease. *J Biol Chem*. 2001;276:36727-36733.
68. Balapattabi K, Little JT, Farmer GE, Cunningham JT. High salt loading increases brain derived neurotrophic factor in supraoptic vasopressin neurones. *J Neuroendocrinol*. 2018;30:e12639.
69. Amabebe E, Robert FO, Obika LFO. Osmoregulatory adaptations during lactation: thirst, arginine vasopressin and plasma osmolality responses. *Niger J Physiol Sci*. 2017;32:109-116.
70. Suzuki K, Koizumi N, Hirose H, et al. Changes in plasma arginine vasopressin concentration during lactation in rats. *Comp Med*. 2000;50:277-280.
71. Swanson LW, Sawchenko PE. Hypothalamic integration: organization of the paraventricular and supraoptic nuclei. *Annu Rev Neurosci*. 1983;6:269-324.
72. Kannan H, Hayashida Y, Yamashita H. Increase in sympathetic outflow by paraventricular nucleus stimulation in awake rats. *Am J Physiol*. 1989;256:R1325-R1330.
73. Martin DS, Haywood JR. Hemodynamic responses to paraventricular nucleus disinhibition with bicuculline in conscious rats. *Am J Physiol*. 1993;265:H1727-H1733.
74. Pyner S, Coote JH. Identification of branching paraventricular neurons of the hypothalamus that project to the rostroventrolateral medulla and spinal cord. *Neuroscience*. 2000;100:549-556.
75. Zhou JJ, Ma HJ, Shao JY, Pan HL, Li DP. Impaired hypothalamic regulation of sympathetic outflow in primary hypertension. *Neurosci Bull*. 2019;35:124-132.
76. Judy WV, Watanabe AM, Henry DP, et al. Sympathetic nerve activity: role in regulation of blood pressure in the spontaenously hypertensive rat. *Circ Res*. 1976;38:21-29.
77. Allen AM. Inhibition of the hypothalamic paraventricular nucleus in spontaneously hypertensive rats dramatically reduces sympathetic vasomotor tone. *Hypertension*. 2002;39:275-280.
78. Li DP, Pan HL. Role of gamma-aminobutyric acid (GABA)a and gabab receptors in paraventricular nucleus in control of sympathetic vasomotor tone in hypertension. *J Pharmacol Exp Ther*. 2007;320:615-626.
79. Anderson EA, Sinkey CA, Lawton WJ, Mark AL. Elevated sympathetic nerve activity in borderline hypertensive humans. Evidence from direct intraneural recordings. *Hypertension*. 1989;14:177-183.
80. Greenwood JP, Stoker JB, Mary DA. Single-unit sympathetic discharge : quantitative assessment in human hypertensive disease. *Circulation*. 1999;100:1305-1310.
81. Mancia G, Grassi G, Giannattasio C, Seravalle G. Sympathetic activation in the pathogenesis of hypertension and progression of organ damage. *Hypertension*. 1999;34:724-728.
82. Ye ZY, Li DP, Byun HS, Li L, Pan HL. Nkcc1 upregulation disrupts chloride homeostasis in the hypothalamus and increases neuronal activity-sympathetic drive in hypertension. *J Neurosci*. 2012;32:8560-8568.
83. Herman JP, Tasker JG. Paraventricular hypothalamic mechanisms of chronic stress adaptation. *Front Endocrinol (Lausanne)*. 2016;7:137.
84. Sunstrum JK, Inoue W. Heterosynaptic modulation in the paraventricular nucleus of the hypothalamus. *Neuropharmacology*. 2018; Nov 5. pii: S0028-3908(18)30841-4. https://doi.org/10.1016/j.neuropharm.2018.11.004. [Epub ahead of print].
85. Roland BL, Sawchenko PE. Local origins of some gabaergic projections to the paraventricular and supraoptic nuclei of the hypothalamus in the rat. *J Comp Neurol*. 1993;332:123-143.
86. Boudaba C, Szabo K, Tasker JG. Physiological mapping of local inhibitory inputs to the hypothalamic paraventricular nucleus. *J Neurosci*. 1996;16:7151-7160.
87. Cole RL, Sawchenko PE. Neurotransmitter regulation of cellular activation and neuropeptide gene expression in the paraventricular nucleus of the hypothalamus. *J Neurosci*. 2002;22:959-969.
88. Maguire J. Stress-induced plasticity of gabaergic inhibition. *Front Cell Neurosci*. 2014;8:157.
89. Hewitt SA, Wamsteeker JI, Kurz EU, Bains JS. Altered chloride homeostasis removes synaptic inhibitory constraint of the stress axis. *Nat Neurosci*. 2009;12:438-443.
90. Kanaka C, Ohno K, Okabe A, et al. The differential expression patterns of messenger rnas encoding k-cl cotransporters (kcc1,2) and na-k-2cl cotransporter (nkcc1) in the rat nervous system. *Neuroscience*. 2001;104:933-946.
91. Gao Y, Zhou JJ, Zhu Y, Kosten T, Li DP. Chronic unpredictable mild stress induces loss of GABA inhibition in corticotrophin-releasing hormone-expressing neurons through nkcc1 upregulation. *Neuroendocrinology*. 2017;104:194-208.

How to cite this article: Kim Y-B, Colwell CS, Kim YI. Long-term ionic plasticity of GABAergic signalling in the hypothalamus. *J Neuroendocrinol*. 2019;31:e12753. https://doi.org/10.1111/jne.12753