INTRODUCTION

Neurotransmitter regulation of the neuroendocrine system has commonly been studied and discussed with regard to the role of neuropeptides and monoamines. Yet, similar to the rest of the central nervous system (CNS), hypothalamic interneuronal communication depends critically on signalling via glutamate and GABA, amino acid transmitters that are near-ubiquitous within the neuroendocrine networks, and specialised GABAergic nerve endings throughout the hypothalamus. Glutamate and GABA not only reach neurones through incoming projections, but also as co-transmitters within neuroendocrine neurones. One prominent example of the latter is the tuberoinfundibular dopamine (TIDA) neurones in the arcuate nucleus (ARC), which control pituitary prolactin release by exerting a tonic inhibitory influence. This powerful inhibition constrains prolactin release to specific reproductive events such as late pregnancy, nursing and a pre-ovulatory surge in the oestrus cycle. In the juvenile male rat, TIDA neurones discharge rhythmically and in synchrony, coordinated through strong electrical gap junction-mediated coupling, as revealed in in vitro studies. Recent work has shown that the temporal properties of this oscillation are subject to ultrashort feedback loop regulation exerted by dopamine acting on autoreceptors at the somatodendritic level of TIDA neurones.

Abstract

The secretion of prolactin from the pituitary is negatively controlled by tuberoinfundibular dopamine (TIDA) neurones. The electrical properties of TIDA cells have recently been identified as a modulatory target of neurotransmitters and hormones in the lactotrophic axis. The role of the GABA$_B$ receptor in this control has received little attention, yet is of particular interest because it may act as a TIDA neurone autoreceptor. Here, this issue was explored in a spontaneously active rat TIDA in vitro slice preparation using whole-cell recordings. Application of the GABA$_B$ receptor agonist, baclofen, dose-dependently slowed down or abolished the network oscillations typical of this preparation. Pharmacological manipulations identify the underlying mechanism as an outward current mediated by G-protein-coupled inwardly rectifying K$^+$-like channels. In addition to this postsynaptic modulation, we describe a presynaptic modulation where GABA$_B$ receptors restrain the release of glutamate and GABA onto TIDA neurones. Our data identify both pre- and postsynaptic modulation of TIDA neurones by GABA$_B$ receptors that may play a role in the neuronal network control of pituitary prolactin secretion and lactation.

KEYWORDS

arcuate nucleus, GIRK channels, inhibition, prolactin, tuberoinfundibular...
In addition to dopamine, TIDA neurones appear to signal via the inhibitory neurotransmitter γ-aminobutyric acid (GABA), as first suggested by the coexistence of immunoreactivity for glutamic acid decarboxylase and tyrosine hydroxylase, enzymes of the GABA and dopamine biosynthetic pathways, respectively.\(^{11,12}\) The GABAergic identity of at least a subpopulation of neuroendocrine dopamine neurones is further supported by subsequent studies using ultrastucture,\(^{13}\) reporter gene expression\(^{14-16}\) and optogenetics.\(^{17}\)

The role of GABA in TIDA neurones is, however, poorly understood. Most studies have focused on possible actions in the pituitary, parallel to the inhibitory influence that dopamine exerts on lactotrophs. Indeed, the GABA\(_{B}\) receptor is expressed in the anterior pituitary gland,\(^{16}\) and application of GABA on isolated pituitaries attenuates prolactin release.\(^{19,20}\) The possibility that GABA can act on auto- or heteroreceptors within the TIDA system to regulate the electrical activity of dopaminergic neurones has received little attention. There is good reason to believe that TIDA neurones are subject to GABAergic modulation of their membrane and network properties, which may play a role in shaping the normal rhythms of prolactin release. Indeed, both mRNA\(^{21,22}\) and protein\(^{23}\) for the metabotropic GABA\(_{A}\) receptor are found in the ARC. Electrophysiological studies have also identified G protein-coupled inwardly rectifying K\(^+\) (GIRK)-like currents, a common target for GABA\(_{B}\) receptor activation in TIDA neurones,\(^{17,24,25}\) and some ARC neurones respond to GABA\(_{A}\) agonists by hyperpolarisation.\(^{26,27}\) Notably, stimulation of GABA\(_{A}\) receptors causes circulating prolactin to rise.\(^{28,29}\) Here, we investigated the effects of modulating the activity of the GABA\(_{A}\) receptor on TIDA neurones aiming to provide a better understanding of the role of GABA in the lactotropic axis.

2 | MATERIALS AND METHODS

2.1 | Animals

Experiments were performed on 21-35 day-old male Sprague-Dawley rats (Charles River Laboratory, Cologne, Germany). Animals were housed under a 12:12 hour light/dark cycle with access to food and water available ad lib. The experiments were conducted in accordance with the European Community Council directive of November 24, 1986 (86/609/EEC) and had received prior approval by the local ethical board, Stockholm's Norra Djurförsöksnämnd.

2.2 | Brain slice preparation

Rats were deeply anaesthetised by an i.p. injection of pentobarbital and decapitated. Brains were quickly dissected and placed in an oxygenated ice-cold sucrose solution containing (mmol L\(^{-1}\)): 213 sucrose, 2.5 KCl, 1.3 NaH\(_2\)PO\(_4\), 26 NaHCO\(_3\), 2 MgSO\(_4\), 2 CaCl\(_2\) and 10 D-glucose. Coronal brain slices (250 μm) containing the dorsomedial ARC were cut on a vibratome (Leica Microsystems, Wetzlar, Germany) and transferred to an incubation chamber containing artificial cerebrospinal fluid (aCSF) containing (mmol L\(^{-1}\)): 127 NaCl, 2 KCl, 1.2 NaH\(_2\)PO\(_4\), 26 NaHCO\(_3\), 1.3 MgSO\(_4\), 2.4 CaCl\(_2\) and 10 D-glucose, continuously oxygenated with 95% O\(_2\)-5% CO\(_2\). After a recovery period of at least 1 hour, slices were transferred to the recording chamber and visualised under the microscope with infrared Nomarski differential interference contrast optics (Axioskop ZFS Plus right; Carl Zeiss, Jena, Germany). During the experiments, slices were submerged and continuously perfused (2-3 mL min\(^{-1}\)) with aCSF at room temperature (22-24°C).

2.3 | Electrophysiology: solutions, data acquisition and analysis

Whole cell voltage clamp and current clamp recordings were obtained from TIDA neurones identified on the basis of their typical electrophysiological signature.\(^{8}\) Recordings were performed with 3-6 MΩ pipettes made from borosilicate glass capillaries (World Precision Instruments, Aston, UK) pulled on a P-97 Flaming/Brown micropipette puller (Sutter, Novato, CA, USA) and filled with internal solution containing K-glutonate-based internal solutions (K-glutonate internal) containing (in mmol L\(^{-1}\)): 140 K-glutonate, 10 KCl, 1 KOH, 1 ethylene glycol tetraacetic acid, 2 Na\(_2\)ATP and 10 Hepes, pH 7.3, 280-290 mOsm. TIDA neurones were recorded during a control period of 10 minutes (control condition) and then tested under bath application of drug. Each slice was subjected to only one application of pharmacological agents. Mean membrane potential (or, when applicable, current) values were determined as the average over 10 seconds sampled before drug application, and at the height of effect induced by the drug. When no effect was evident, signal was sampled with a delay similar to the delay observed to obtain full TTX effect. Oscillation frequency was obtained as the inverse of the time to complete five full oscillation cycles (UP + DOWN state), divided by five. Access resistance was monitored throughout the experiments, and neurones in which the series resistance exceeded 15 MΩ or changed ≥ 20% were excluded from the statistics. Liquid junction potential was 16.4 mV and was not compensated.

The recorded signal was amplified using a Multiclamp 700B amplifier (Molecular Devices) and low-pass filtered on-line at 10 kHz, digitised (Digidata 1322A; Molecular Devices, Sunnyvale, CA, USA) and stored on a personal computer using Clampex, version 9.2 (Molecular Devices). Slow and fast capacitive components were automatically compensated. Off-line data analysis and figure preparation were performed with Clampfit, version 9 (Molecular Devices), PRISM (GraphPad Software Inc., San Diego, CA, USA), COREL DRAW (Corel, Ottawa, ON, Canada), and WINEDR and WINWCP (http://spider.science.strath.ac.uk/sipbs/software_ses.htm).

2.4 | Drugs

Baclofen, gabazine, CGP55845, 6-cyano-7-nitroquinoloxaline-2,3-dione (CNQX) and 2-amino-5-phosphonopentanoic acid (AP5) were
purchased from Tocris (Bristol, UK). N-ethylmaleimide (NEM) and barium chloride were purchased from Sigma (St Louis, MO, USA). Tetrodotoxin (TTX) was purchased from Alomone Labs (Jerusalem, Israel). All stock drugs were prepared in water except for CNQX, CGP55845, and NEM (DMSO). All stock solutions were then diluted to their final concentration in aCSF.

2.5 Statistical analysis

Statistical significance for all analyses was determined using a two-tailed Student’s t test in PRISM (unpaired when comparing two populations of cells and paired when comparing conditions in the same cell population). All data values are reported as the mean ± SEM unless otherwise indicated. P < 0.05 was considered statistically significant.

3 RESULTS

3.1 GABAB receptors can abolish or slow down TIDA oscillations depending on concentration

Input resistance was determined in slices exposed to tetrodotoxin (TTX) (500 nmol L⁻¹), a treatment that abolishes TIDA oscillations, likely as a result of the blocking actions of this drug on the persistent Na⁺ current. Application of baclofen (1 µmol L⁻¹) resulted in a decrease in input resistance (Control: 1547 ± 76 MΩ; baclofen: 1376 ± 69 MΩ; n = 5 cells; P = 0.009; paired t test) (Figure 1Eb,c), as determined by negative square pulse current injections, indicating the opening of membrane channels in response to GABAB receptor stimulation. Under these conditions, a significant hyperpolarisation of the resting membrane potential was also observed (TTX: −44.7 ± 0.75 mV; TTX and Baclofen: 1 µmol L⁻¹: −50.7 ± 2 mV; n = 5 cells; P = 0.033; paired t test) (Figure 1Ed).

3.2 Postsynaptic GABAB actions on TIDA neurones are mediated by GIRK channels

To further characterise the membrane actions of baclofen on TIDA neurones that underlie the effect on oscillations, we applied the drug during voltage clamp recordings in the presence of TTX and at a command potential (Vcommand) of −60 mV. Following the application of baclofen (10 µmol L⁻¹), a reversible net outward current (Ibaclofen) of 18.9 ± 3.1 pA (Holding current control: −18.9 ± 3.6 pA vs in baclofen: 0.3 ± 3.9 pA; n = 11 cells; P ≤ 0.001; paired t test) was observed (Figure 2Aa,b). GABAB receptors are G protein-coupled receptors that activate different downstream effectors. The best characterised of these are the G protein-coupled inwardly rectifying K⁺ (GIRK) channels. We performed a series of voltage clamp experiments to determine whether opening of GIRK channels could underlie the baclofen actions observed in TIDA neurones. In voltage clamp ramp recordings (interval −120-0 mV) (Figure 2Ad) performed in the presence of TTX, subtraction of the traces recorded before and during application of baclofen (10 µmol L⁻¹) yielded a net outward current (Ibaclofen) with a reversal potential (extrapolated from the linear portion of the IV curve) of approximately −123 mV (Figure 2Ae), a value that is lower than that calculated for K⁺. This hyperpolarised value is best explained by mediation via K⁺ flux. To determine whether opening of GIRK channels could underlie the baclofen actions observed in TIDA neurones. In voltage clamp ramp recordings (interval −120-0 mV) (Figure 2Ad) performed in the presence of TTX, subtraction of the traces recorded before and during application of baclofen (10 µmol L⁻¹) yielded a net outward current (Ibaclofen) with a reversal potential (extrapolated from the linear portion of the IV curve) of approximately −123 mV (Figure 2Ae), a value that is lower than that calculated for K⁺. This hyperpolarised value is best explained by mediation via K⁺ flux.

Next, we explored the sensitivity of the TIDA neurone to Ba²⁺, a wide spectrum blocker of inwardly rectifying K⁺ channels. In the presence of Ba²⁺ (300 µmol L⁻¹), the inward current induced by baclofen (10 µmol L⁻¹) at Vcommand = −60 mV (and in the presence of TTX) was diminished by 47.4 ± 7.5% (Control: 16.8 ± 2 pA; Baclofen: 10 µmol L⁻¹, 9.1 ± 2 pA; n = 5 cells; P = 0.001; paired t test) (Figure 2Ba,b). Finally, we tested whether Ibaclofen requires the binding of G-proteins by using the sulfhydryl alkylating agent NEM, which uncouples G-protein from receptors. Recording of TIDA neurones at Vcommand = −60 mV in the presence of TTX revealed a dramatic reduction (by 92 ± 3%) of Ibaclofen during application of NEM (Control: 22.4 ± 4.1 pA; Baclofen: 10 µmol L⁻¹, 1.4 ± 0.4 pA; n = 5 cells; P = 0.009; paired t test) (Figure 2Ca,b). Taken together,
Baclofen 10 µM

Baclofen 0.1 µM

Baclofen 0.01 µM

TTX 500 nM

Control

Baclofen

V_m (mV)

Control

Baclofen

V_m (mV)

Input Resistance (MΩ)

Oscillation frequency (Hz)

TTX

Baclofen
the hyperpolarised reversal potential and Ba^{2+}- and NEM-sensitivity indicate that f_{baclofen} is mediated through GIRK-like channels.

### 3.3 | Little evidence for endogenous GABA\textsubscript{B} activation in vitro

The spontaneously active TIDA preparation\(^8\) presents an opportunity to study the role of endogenously released transmitters within the slice, as is the case for e.g. dopamine.\(^10\) To evaluate the possibility of ongoing GABA\textsubscript{B} receptor activation resulting from GABA release from TIDA neurones and/or other cells within the slice, we applied CGP55845, a selective GABA\textsubscript{B}-type antagonist.\(^39\) GABA\textsubscript{B} blockade (10 \(\mu\text{mol L}^{-1}\) CGP55845) failed to alter oscillation frequency (Control: 0.055 ± 0.004 Hz; CGP: 0.054 ± 0.003 Hz; \(n = 9\) cells; \(P = 0.621\); paired t test) (Figure 3A,B). We also analysed the effect of GABA\textsubscript{B} blockade on membrane voltage (measured as nadir potential). The majority of cells (6/9 cells) responded by depolarisation to application of CGP55845 (10 \(\mu\text{mol L}^{-1}\)), whereas the remaining cells (3/9 cells) responded by hyperpolarisation; the net population effect was not significant (Nadir control: −73.3 ± 3.6 mV in CGP55845 (10 \(\mu\text{mol L}^{-1}\)); −72.3 ± 3.4 mV; \(n = 9\) cells; \(P = 0.475\); paired t test) (Figure 3C). All together, these data suggest an absence of tonic GABA\textsubscript{B} receptor-mediated tonic inhibition in the in vitro preparation.

### 3.4 | GABA\textsubscript{B} receptor-mediated inhibition of excitatory and inhibitory synaptic transmission

Finally, we addressed whether GABA\textsubscript{B} receptors, in addition to their postsynaptic actions (vide supra), can also exert a presynaptic influence on transmitter release onto TIDA neurones. In other neuronal populations, GABA\textsubscript{B} receptors have been shown to control presynaptic calcium channels.\(^40,41\) Thus, we first isolated spontaneous inhibitory postsynaptic currents (sIPSCs) by application of the glutamate AMPA/kainate and NMDA receptor antagonists, CNQX (10 \(\mu\text{mol L}^{-1}\)) and AP5 (50 \(\mu\text{mol L}^{-1}\)), respectively, and using a high [Cl\textsuperscript{-}]

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**FIGURE 1** Application of the GABA\textsubscript{A} agonist, baclofen, slows down or abolishes tuberoinfundibular dopamine (TIDA) oscillations in a concentration-dependent manner. Aa. Current clamp recording of a TIDA neurone in vitro. Application of 10 \(\mu\text{mol L}^{-1}\) baclofen results in hyperpolarisation of TIDA membrane potential and reversible abolition of phasic discharge. Dashed line in red represents membrane potential at −60 mV in all represented recordings. Ab. Quantification of recordings as that shown in Aa (grey lines represent individual data points; black line represents the mean ± SEM; \(n = 5\) cells, \(P = 0.002\), paired t test). B. Application of 1 \(\mu\text{mol L}^{-1}\) baclofen during a current clamp recording of an oscillating TIDA neurone results in a complete silencing of the oscillating activity and depolarisation of TIDA membrane potential compared to the oscillation nadir. C. In the presence of 0.1 \(\mu\text{mol L}^{-1}\) baclofen, the oscillation frequency of TIDA neurones slows down (73.2 ± 3.4 mV; Baclofen: 55.8 ± 7.5 mV; \(n = 5\) cells; \(P = 0.003\); paired t test), which is not associated with a change in the membrane potential of TIDA neurones. Cb. Superimposed black (control) and grey (0.1 \(\mu\text{mol L}^{-1}\) baclofen) traces at increased temporal resolution. Cc. Quantification of the effect illustrated as in Ab (\(n = 7\) cells, \(P = 0.009\), paired t test). Da. In the presence of 0.01 \(\mu\text{mol L}^{-1}\) baclofen, no change is observed in TIDA oscillation frequency (Control: 5.0 ± 0.8289 mV; Baclofen: 5.0 ± 0.585 mV; \(n = 5\) cells; \(P = 0.8289\); paired t test) or membrane potential (\(n = 5\) cells, \(P = 0.585\); paired t test). Db. Superimposed black (control) and grey (0.01 \(\mu\text{mol L}^{-1}\) baclofen) traces at increased temporal resolution. Dc. Quantification of the effect illustrated as in (Ab). Ea. A TIDA neurone recorded in current clamp mode in the presence of tetrodotoxin (TTX). Application of baclofen (1 \(\mu\text{mol L}^{-1}\)) results in a hyperpolarisation. To compare change in input resistance under identical membrane potential, negative constant current is injected (−20 pA, 500 ms). Input resistance was tested before (*) and during application of baclofen (*). Eb. Illustration of the test pulse protocol and the voltage response (Control: black trace; baclofen: grey trace). Ec. Application of baclofen results in a decrease of input resistance (From 1547 ± 76 MΩ to 1376 ± 69 MΩ) (\(n = 5\) cells, \(P = 0.011\), paired t test). Ed. Quantification of the baclofen-induced hyperpolarisation in the presence of TTX. *P < 0.05, **P < 0.01, ns, not significant.
FIGURE 2  Stimulation of GABAβ receptors on tuberoinfundibular dopamine (TIDA) neurones activates G protein-coupled inwardly rectifying K⁺ (GIRK)-like channels. Aa, Voltage clamp recording of a TIDA neurone in vitro clamped at −60 mV. After application of TTX (500 nmol L⁻¹; resulting in a cessation of oscillations), subsequent application of baclofen (10 µmol L⁻¹) yields a reversible outward current (I_Baclofen). Dashed line in red represents 0 pA holding current. Ab, Mean peak current amplitude in control and in the presence of baclofen (grey lines represent individual data points; black line represents the mean ± SEM). Ac, Averaged holding current frequency distribution from recordings as that illustrated in Aa; in control (black) and in the presence of baclofen (red; n = 11 cells). Ad, Voltage clamp ramp protocol to extract I_Baclofen over a spectrum of membrane potentials. Ae, Averaged baclofen induced current obtained by the digital subtraction of voltage-clamp ramps performed in control and at peak of baclofen response (n = 5 cells). The estimated reversal potential (E_rev) of the I_Baclofen is −123.4 mV (obtained by linear fit of I_Baclofen). Ba, Voltage clamp recording of a TIDA neurone clamped at −60 mV in the presence of TTX. In the presence of the non-specific GIRK channel blocker, Ba²⁺ (300 µmol L⁻¹), I_Baclofen is significantly decreased. Bb, Quantification of recordings as that shown in (Ba) (grey lines represent individual data points; black line represents the mean ± SEM; n = 5 cells, P = 0.001, paired t test). Ca, TIDA neurone recorded in voltage clamp mode in the presence of TTX (command potential −60 mV). Application of the G-protein uncoupling agent, N-ethylmaleimide (NEM) (250 µmol L⁻¹), induces a reduction of I_Baclofen. Cb, Quantification of the NEM effect illustrated as in (Ab) (n = 5 cells, P = 0.009, paired t test). **P < 0.01, and ***P < 0.001

A

B

C
The sEPSC frequency was significantly attenuated during baclofen application in both the UP (Control: 2.5 ± 0.6 Hz; Baclofen: 1.1 ± 0.3 Hz; n = 6 cells; P = 0.016; paired t test) (Figure 4Be) and the DOWN (Control: 2.3 ± 0.6 Hz; Baclofen: 1.1 ± 0.3 Hz; n = 6 cells; P = 0.036; paired t test) (Figure 4Bf) states. Taken together, these data indicate a potent GABA<sub>B</sub>-mediated modulation of glutamatergic and GABAergic pre-synaptic release onto TIDA neurones, and that this effect does not depend on oscillation state.

**4 | DISCUSSION**

The pharmacological control of the neuroendocrine system has primarily been studied in the context of neuropeptidergic and monoaminergic modulation. With the exception of the gonadotrophic axis, the role of GABA and glutamate has received comparatively little attention, even though they constitute the two dominant transmitters in the CNS. Here, we have focused on the GABA<sub>B</sub> receptor in the lactotrophic axis, more specifically in the TIDA population. By using a spontaneously active rat preparation, we show that stimulation of GABA<sub>B</sub> receptors by baclofen dose-dependently slows down and, at higher concentrations, abolishes network oscillations, whereas TIDA neurones appear to be electrophysiologically insensitive to GABA<sub>B</sub> antagonism in vitro. The GABA<sub>B</sub>-dependent effect on network rhythms is associated with a hyperpolarisation that bears the pharmacological hallmarks of GIRK currents. Furthermore, we demonstrate a generalised attenuation of PSCs when GABA<sub>B</sub> receptors are activated. These
findings suggest that metabotropic GABA receptors can modulate TIDA activity, and thus, by inference, contribute to the regulation of prolactin secretion.

The suppressive action on network rhythms is partly underpinned by a postsynaptic effect involving an outward current. The reversal of $I_{\text{baclofen}}$ at ca. $-123 \text{ mV}$ supports $K^+$ as a charge carrier. (This value is more hyperpolarised than the calculated reversal potential for $K^+$ at $-111 \text{ mV}$, although it may be explained by the presence of strong gap junction coupling in the rat TIDA network, which negatively impacts on space clamp.) The partial sensitivity of $I_{\text{baclofen}}$ to $Ba^{2+}$ suggests that it corresponds to an inwardly rectifying $K^+$ current (but that additional, as yet unidentified, currents may also contribute). The failure of baclofen to elicit an outward current in the presence of NEM indicates that $I_{\text{baclofen}}$ is dependent on intact G-protein signalling. Combined, these findings offer strong evidence that GABA$_B$ receptors activate a GIRK current in TIDA neurones, as has been shown elsewhere in the CNS. Indeed, GIRK channels have been demonstrated as targets of $\mu$-opioid receptors and serotonin (via the 5-HT$_{1A}$ receptor) on TIDA neurones. Earlier work has also reported the presence of the GABA channels and baclofen-mediated hyperpolarisation in the arcuate nucleus. Thus, GIRK channels may form a pharmacological target on TIDA cells that integrate the actions of several neuromodulators in the control of prolactin secretion. It should be noted, however, that there may be divergence of signalling pathways that rely on GIRK activation: Wagner et al. demonstrated that tolerance to opioid stimulation (which also involves GIRK channels) does not cross-transmit to GABA$_B$ signalling in TIDA neurones.

Furthermore, the present findings add the GABA$_B$ receptor to a growing list of modulators that can act on TIDA membrane properties to switch the network behaviour of these neurones. Previously identified members of this group include (in addition to those mentioned above) oxytocin, thyrotropin-releasing hormone and orexin/hypocretin, as well as prolactin and dopamine. In the latter case, the actions may reflect an autoinhibitory short-feedback loop. The GABA$_B$ actions described here may also form part of feedback inhibition as TIDA neurones express several GABAergic markers. Optogenetically based experiments have suggested the existence of recurrent GABAergic autoinnervation within this population. Furthermore, GABA$_B$-mediated inhibition has been described in ARC GABAergic neurones of ovariecotomised guinea pigs, which may include the TIDA neurones. However, since GABA is a near-ubiquitous inhibitory transmitter, it is likely that TIDA neurones receive GABAergic afference from multiple (also non-TIDA) sources. The possible contribution of autoinnervation to GABA$_B$ actions on TIDA neurones remains to be determined.

Although the responses to baclofen were robust, we failed to observe any modulation of TIDA oscillation properties in the presence of CGP55845. (It should be noted that we did not specifically examine the effect of blocking GABA$_B$ receptors on synaptic input to TIDA neurones. Yet, absent an effect on oscillation behaviour in these cells, it appears unlikely that any potential modulation of synaptic currents would impact significantly on the overall electrical state of TIDA neurones.) These observations may suggest that GABA$_B$ receptors on TIDA neurones are not activated under baseline conditions, at least not in the ex vivo preparation used in the present study. GABA$_B$ receptors typically exhibit an extrasynaptic distribution and activate only with sufficient spillover from synaptically released GABA as in the hippocampus and cerebellum. Thus, GABA$_B$ receptors might be activated and impact on TIDA neurone physiology only under conditions of more intense GABAergic transmission. In the scenario above, where GABA$_B$ receptors may be autoreceptors, they could form part of an autoregulatory loop in the event of excess neuronal activity. In support of this hypothesis, central administration of baclofen decreases dopamine release in the median eminence, where neuroendocrine TIDA terminals are found.

In addition to the postsynaptic effects, we also found that a decrease in the frequency of both excitatory and inhibitory synaptic input to TIDA neurones in the presence of baclofen. The role of presynaptic GABA$_B$ receptors regulating both excitatory and inhibitory impulse traffic is well documented in other CNS regions. Thus, the overall effect of baclofen on TIDA network activity may also include actions at the terminal level. In the intact brain, postsynaptic (somatodendritic) and presynaptic (terminal) GABA$_B$ receptors could be activated in isolation (in contrast to the in vitro...
bath application conditions), allowing for a fine-tuning at separate cell compartments.

In the present study, we focused on the peripubertal male TIDA system, where the dopaminergic brake on prolactin release is assumed to be less subject to fluctuation than in the female where prolactin rises transiently during pro-oestrus/oestrus and more persistently during lactation. Whether GABA 2 receptors constitute a brake on TIDA neurons, potentially acting as autoreceptors providing inhibition. Postsynaptic GABA 2 receptors inhibit TIDA network oscillations and action potential discharge via G-protein-coupled inwardly rectifying K+ (GIRK) channels. Presynaptic GABA 2 receptors inhibit transmitter release from glutamatergic and GABAergic terminals onto TIDA neurons.

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**CONFLICT OF INTERESTS**

The authors declare that they have no conflicts of interest.

**AUTHOR CONTRIBUTIONS**

RA and CB designed the experiments and wrote the manuscript. RA performed and analysed all experiments.

**DATA AVAILABILITY**

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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**FIGURE 5** The role of GABA 2 receptors in tuberoinfundibular dopamine (TIDA) neurones as suggested by the current results. A, Organisation of the hypothalamo–pituitary lactotrophic axis. TIDA neurones in the arcuate nucleus form a gap junction-coupled network that provides tonic inhibition (‘STOP’) of pituitary prolactin release into the systemic circulation, via neurohemal release of dopamine in the median eminence. B, GABA 2 receptors constitute a brake on TIDA neurones, potentially acting as autoreceptors providing inhibition. Postsynaptic GABA 2 receptors inhibit TIDA network oscillations and action potential discharge via G-protein-coupled inwardly rectifying K+ (GIRK) channels. Presynaptic GABA 2 receptors inhibit transmitter release from glutamatergic and GABAergic terminals onto TIDA neurones. 3V, third ventricle; DA, dopamine; PRL, prolactin.
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