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SHORT COMMUNICATION

Dopaminergic inhibition of gonadotropin-releasing hormone neurons in the cichlid fish *Astatotilapia burtoni*

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ABSTRACT

Dopamine regulates reproduction in part by modulating neuronal activity within the hypothalamic-pituitary–gonadal (HPG) axis. Previous studies suggested numerous mechanisms by which dopamine exerts inhibitory control over the HPG axis, ultimately changing the levels of sex steroids that regulate reproductive behaviors. However, it is not known whether these mechanisms are conserved across vertebrate species. In particular, it is unknown whether mechanisms underlying dopaminergic control of reproduction are shared between mammals and teleost fish. In mammals, dopamine directly inhibits gonadotropin-releasing hormone (GnRH1) hypothalamic neurons, the gatekeepers for activation of the HPG axis. Here, we demonstrate, for the first time in teleost fish, dopaminergic control of GnRH1 neurons via direct dopamine type-2-like receptor (D2R)-mediated inhibition within the hypothalamus. These results suggest that direct dopaminergic control of GnRH1 neurons via interactions in the hypothalamus is not exclusive to tetrapod reproductive control, but is likely conserved across vertebrate species.

KEY WORDS: GnRH, HPG axis, Hypothalamus, Dopamine, Reproduction

INTRODUCTION

Dopamine regulates a variety of physiological and behavioral processes, including reproduction, which, in vertebrates, is controlled via the hypothalamic-pituitary–gonadal (HPG) axis. Hypothalamic release of gonadotropin-releasing hormone (GnRH1, previously called luteinizing hormone-releasing hormone), stimulates pituitary gonadotropic cells to release luteinizing hormone (LH) and follicle-stimulating hormone (FSH) into the bloodstream. These gonadotropic hormones directly alter reproductive potential by driving the synthesis of gonadal steroid hormones including testosterone, estrogen and progestin. In tetrapods, GnRH1 reaches the pituitary via a specialized portal vasculature, while in fish it arrives directly via neuronal connections (Dufour et al., 2010).

Dopamine likely influences the HPG axis primarily at the level of the hypothalamus and pituitary, by regulating the release of the gonadotropic hormones (Dufour et al., 2005). However, the mechanisms underlying dopaminergic regulation of gonadotropic hormone release remain unclear, particularly in fish. Previous studies demonstrate that in tetrapods, dopamine inhibits GnRH1 release (Ching and Lin, 1994; Lacau-Mengido et al., 1993; Owens et al., 1980; Tasaka et al., 1985) via actions at both the hypothalamic soma of GnRH1 neurons and their synaptic terminals within the portal vasculature (Contijoch et al., 1992; Corio et al., 1990; Kuljis and Advis, 1989; Lehman et al., 1988; Liu et al., 2013; Pehrson et al., 1983; Ugrumov et al., 1989). In fish, dopamine was thought to act primarily within the pituitary, reducing gonadotropin release either indirectly, by suppressing GnRH1-releasing axons, or directly, by inhibiting pituitary gonadotropes (Levavi-Sivan et al., 1995; Vacher et al., 2002; Van Goor et al., 1998; Yu and Peter, 1992; Yu et al., 1991). Although early studies suggested a hypothalamic site of dopaminergic GnRH1 inhibition in fish (Yu and Peter, 1992; Yu et al., 1991), it remained unknown whether dopamine directly interacts with GnRH1 neurons within the hypothalamic compartment. Here, we demonstrate, for the first time in teleost fish, that dopamine inhibits GnRH1 cells within the hypothalamus via selective activation of dopamine type-2 receptors.

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MATERIALS AND METHODS

Animals

Cichlid fish, *Astatotilapia burtoni* (Günther 1894), were derived from animals collected in Lake Tanganyika, East Africa (Fernald and Hirata, 1977). Some fish carried a *GnRH1:eGFP* transgene producing enhanced green fluorescent protein (eGFP) in all GnRH1 neurons (Ma et al., 2015; White et al., 1995). Animals were maintained in aquaria under conditions mimicking their native habitat (pH 8.0, 26–28°C, 12 h light:12 h dark cycle). Aquaria contained gravel and pots to facilitate territory establishment. Experiments were conducted in compliance with guidelines of the Stanford Institutional Animal Care and Use Committee.

Immunohistochemistry

Brains were dissected, fixed for 2 h in 4% paraformaldehyde then sunk overnight in 30% sucrose dissolved in PBS (0.1 mol l^{-1}). Brains were embedded in Neg50 (Thermo Fisher Scientific, Waltham, MA, USA) and cryosectioned to 30 µm (Microm HM 550, Zeiss, Oberkochen, Germany). Slides were incubated in PBS+0.1% Triton X-100 with 1% goat serum, then exposed to primary antibodies overnight at 4°C, diluted in PBS+0.1% Triton X-100 with 0.1% goat serum (PGX): chicken anti-eGFP [1:500; ab13970 (lot GR236651-4), Abcam, Cambridge, UK] and mouse anti-tyrosine hydroxylase (TH, 1:500; 22941, ImmunoStar, Hudson, WI, USA). Secondary antibodies in PGX were applied for 1 h at 23°C: FITC goat anti-chicken [1:300; F-1005 (lot FGC949388), Aves Labs, Tigard, OR, USA] and AlexaFluor 594 goat anti-mouse [1:300; 115-585-003 (lot 12500), Jackson ImmunoResearch, West Grove, PA, USA]. Images were acquired with a Zeiss LSM 700 confocal microscope and processed using ImageJ (NIH).

In vitro slice preparation and recordings

To record from GnRH1 neurons in the preoptic area (POA), brain slices were prepared. For experiments shown in Figs 2A,B and 3A,B, slices were prepared as previously described (Greenwood and

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Fernald, 2004). For all other electrophysiology experiments, male and female *GnRH1:eGFP*+ transgenic fish, >15–20 weeks old, were decapitated and the brains transferred to ice-cold (4°C), oxygenated (95% O_2, 5% CO_2) slicing solution containing (in mmol l^{-1}): 234 sucrose, 11 glucose, 24 $NaHCO_3$, 2.5 KCl, 1.25 $NaHPO_4$, 10 $MgSO_4$ and 0.5 $CaCl_2$, embedded in 4% low melting point agarose, rapidly cooled then mounted on a vibratome (VT1200, Leica Microsystems, Buffalo Grove, IL, USA); 250 µm-thick slices containing the POA were collected from the ventral surface of the brain (Greenwood and Fernald, 2004). Slices were incubated in 34°C, oxygenated ACSF, containing (in mmol l^{-1}): 10 glucose, 26 $NaHCO_3$, 1.25 $NaHPO_4$, 2.5 KCl, 1 $MgSO_4$, 2 $CaCl_2$ and 126 NaCl (298 mOsm) for 30 min, then incubated at 23°C for >30 min before recording. Slices were perfused in a submerged chamber with ACSF at a rate of 2–3 ml min^{-1}. For experiments shown in Figs 2A,B and 3A,B, recordings were conducted as previously described (Greenwood and Fernald, 2004); recorded neurons were filled with biocytin and identified as GnRH1-expressing cells via *post hoc* immunostaining. For all other experiments, POA GnRH1:eGFP+ cells were identified by epifluorescence illumination. Borosilicate glass pipettes (6–12 mΩ) were filled with potassium gluconate internal solution containing (in mmol l^{-1}): 130 potassium gluconate, 10 KCl, 10 Hepes, 10 EGTA, 2 NaCl, 4 MgATP and 0.3 NaGTP. Signals were amplified with a Multiclamp 700B, digitized by a Digidata 1400 at 20 kHz, and acquired with pClamp10 (Molecular Devices, Sunnyvale, CA, USA).

Pharmacology

Drugs were dissolved in ACSF to the following final concentrations: dopamine, 1, 10 or 100 µmol l^{-1}; SKF81297, 10 µmol l^{-1}; quinpirole, 10 µmol l^{-1}; cadmium chloride ($CdCl_2$), 100 µmol l^{-1}; and tetrodotoxin (TTX), 0.5 µmol l^{-1}.

Data analysis

Data were analyzed with custom-written Matlab (MathWorks, Natick, MA, USA) scripts (available upon request). For drug wash-on experiments, baseline activity was recorded in current-clamp mode for 0.33–2.5 min, then slices were exposed to drugs for 1–4 min. Control activity reflects the mean membrane potential (V_m) during the baseline period; drug activity reflects mean membrane potential following drug wash-on. For experiments shown in Fig. 3A,B, slices were pre-incubated in $CdCl_2$ or TTX for >15 min. For those shown in Fig. 3C,D cells were presented with a series of 10 pA steps, from −100 to 0 pA; data are reported as the mean steady-state voltage response induced by current injection, calculated by subtracting pre-injection resting voltage (V_{rest}) from membrane voltage during current injection. For population averages, median values and interquartile ranges (IQRs) are reported. Statistical analyses and power calculations were performed using Prism (Graphpad, La Jolla, CA, USA).

RESULTS AND DISCUSSION

Dopamine controls vertebrate reproduction by inhibiting the release of pituitary gonadotropins. In fish, most studies report the site of dopaminergic inhibition of gonadotropin release as within the pituitary itself, via either inhibition of GnRH1-releasing axons or suppression of gonadotropin-releasing cells. In tetrapods, an additional site is described at the soma of hypothalamic GnRH1 neurons located in the POA. We asked: is direct dopaminergic control of GnRH1 neurons via interactions within the POA exclusive to tetrapod reproduction control, or is it a mechanism found across vertebrate species?

Local TH-positive processes within the POA

We first looked for the presence of dopaminergic fibers in the hypothalamus of *A. burtoni*. Previously, dopaminergic cell bodies were observed near the POA in several teleost fish species, including *A. burtoni* (Goebrecht et al., 2014; O'Connell et al., 2010; Saha et al., 2015). We tested whether dopaminergic processes are closely apposed to GnRH1 neurons. We immunostained hypothalamic sections from *GnRH1:eGFP* fish for GFP to label GnRH1 neurons and TH, a marker of dopaminergic neurons. We observed many TH-positive processes in close proximity to GnRH1 neurons (Fig. 1, upper row) and TH-positive cell bodies near GnRH1 soma (Fig. 1, lower row, arrowhead). These cell bodies imply a local source of TH-positive processes, although external sources, as reported in mammals (Miller and Lonstein, 2009), may contribute. Our findings agree with previous reports of direct contact between dopaminergic terminals and GnRH1 cells in mammals (Jennes et al., 1983; Lehman et al., 1988; Leranth et al., 1988; Pompolo et al., 2003; Tillet et al., 1989). Together, they suggest local dopamine within the hypothalamus could be a conserved mechanism of reproductive control across a broad array of vertebrates.

Dopamine inhibits GnRH1 neurons

To test whether dopamine influences the activity of *A. burtoni* GnRH1 neurons in the POA, we isolated the soma of GnRH1 neurons by cutting acute brain slices of the POA, which excluded two previously identified sites of dopaminergic control of gonadotropin release – pituitary GnRH1 axonal terminals and gonadotropes (Levavi-Sivan et al., 1995; Vacher et al., 2002; Van Goor et al., 1998; Yu and Peter, 1992; Yu et al., 1991). We bath applied dopamine (1–100 µmol l^{-1}) while performing current-clamp recordings of GnRH1 neurons. Dopamine strongly hyperpolarized GnRH1 neurons at all concentrations [Fig. 2A, example trace; Fig. 2B, population; control V_m: −51.3 mV, IQR −55.4 to −48.0 mV; dopamine V_m: −67.2 mV, IQR −73.9 to −59.0 mV; N=6; P=0.03, two-tailed Wilcoxon matched-pairs signed rank test (Wilcoxon t-test)]. These results demonstrate that dopamine drives inhibition of GnRH1 neurons in *A. burtoni* POA.

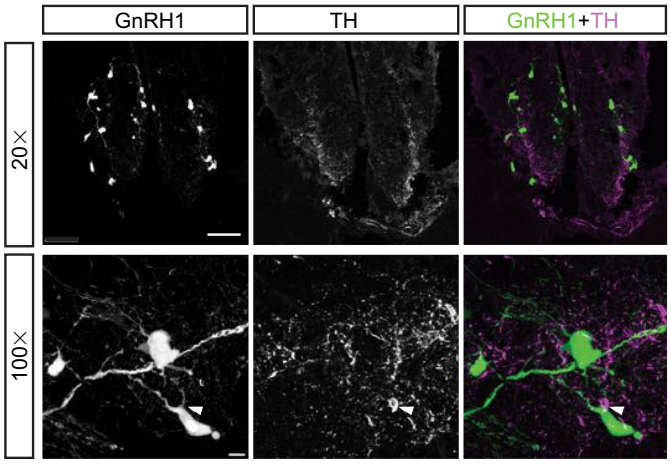

Fig. 1. Presence of dopaminergic processes in the preoptic area (POA). Upper row: maximum projection confocal image of eGFP-labeled gonadotropin-releasing hormone (GnRH1) neurons (green) surrounded by anti-tyrosine hydroxylase (TH)-immunostained processes and cell bodies (magenta). Staining replicated across three fish. Dorsal is up. Scale bar, 100 µm. Lower row: high magnification maximum projection confocal image showing close apposition of eGFP-expressing GnRH1 neurons and a TH-immunostained neuron (arrowhead). Scale bar, 10 µm.

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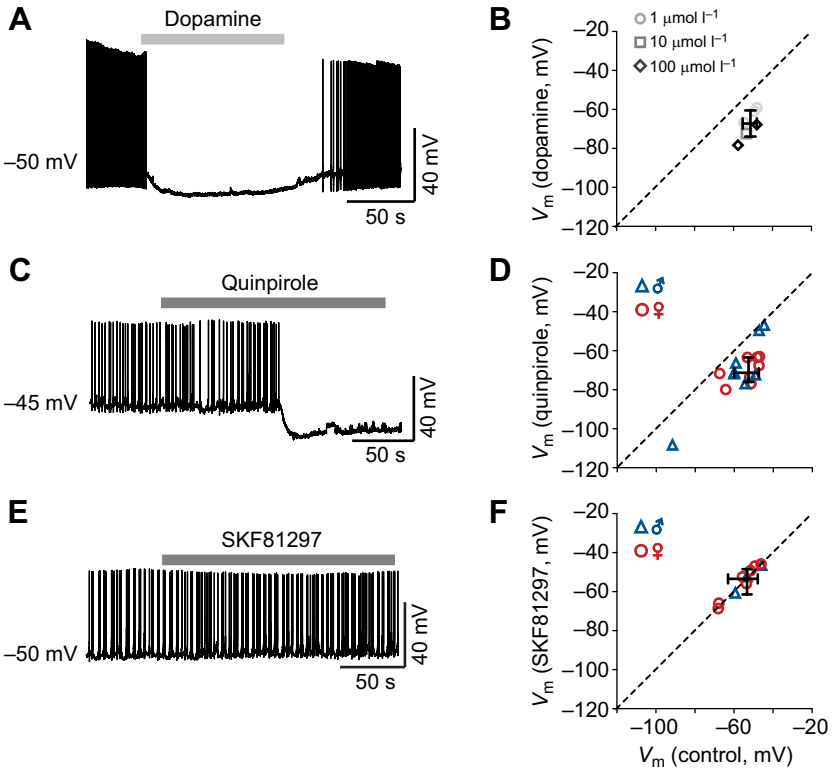

Fig. 2. Dopamine and dopamine type-2-like receptor (D2R) agonists hyperpolarize GnRH1 neurons in the POA. (A) Example intracellular recording from a tonically firing GnRH1 neuron; bath application of dopamine (1 µmol l^{-1}; gray bar) hyperpolarizes the resting membrane potential (V_m) below threshold. (B) Bath application of 1, 10 or 100 µmol l^{-1} dopamine hyperpolarizes GnRH1 neurons (N=6 neurons, P=0.03, two-tailed Wilcoxon t-test). Neurons were recorded from brain slices collected from five fish. Values are the mean steady-state voltage response for each neuron. The dashed line indicates no change after drug application (line of unity). The crosshair represents the median effect (center) for the population, with interquartile range (IQR). (C) Example intracellular recording from a GnRH1 neuron. Bath application of quinpirole (gray bar), a D2R-specific agonist, drives neuronal hyperpolarization. (D) Summary of the effect of quinpirole application on female and male GnRH1 neurons (N=16 neurons, P=0.0005, two-tailed Wilcoxon t-test). Each neuron was recorded from a unique brain slice, collected from 13 fish. Conventions as for B. (E) Bath application of SKF81297 (gray bar), a D1 receptor-specific agonist, does not elicit any change in resting membrane potential in an intracellular recording from a representative GnRH1 neuron. (F) Summary of the effect of SKF81297 application on female and male GnRH1 neurons (N=10 neurons, P=0.32, two-tailed Wilcoxon t-test). Each neuron was recorded from a unique brain slice, collected from eight fish. Conventions as for B.

Selective D2, but not D1, receptor agonists inhibit GnRH1 neurons

To elucidate the cellular and circuit mechanisms underlying dopaminergic inhibition of GnRH1 neurons, we next asked which dopamine receptor types regulate GnRH1 neurons. Bath application of quinpirole (10 µmol l^{-1}), a selective D2-like receptor (D2R) antagonist, strongly hyperpolarized GnRH1 neurons (Fig. 2C, example trace; Fig. 2D, population; control: −52.6 mV, IQR −60.1 to −63.4 mV; quinpirole: −71.5 mV, IQR −76.0 to −63.4 mV; N=16; P=0.0005, Wilcoxon t-test; Fig. S1: all traces). In contrast, bath application of SKF81297 (10 µmol l^{-1}), a selective D1 receptor antagonist, had no effect on GnRH1 neuronal membrane potentials (Fig. 2E,F; control: −53.5 mV, IQR −61.5 to −48.5 mV; SKF81297: −52.3 mV, IQR −62.0 to −46.8 mV; N=10; P=0.32, Wilcoxon t-test). These results suggest that dopamine-mediated GnRH1 cell inhibition is accomplished via D2Rs, and not D1-like receptors (D1Rs).

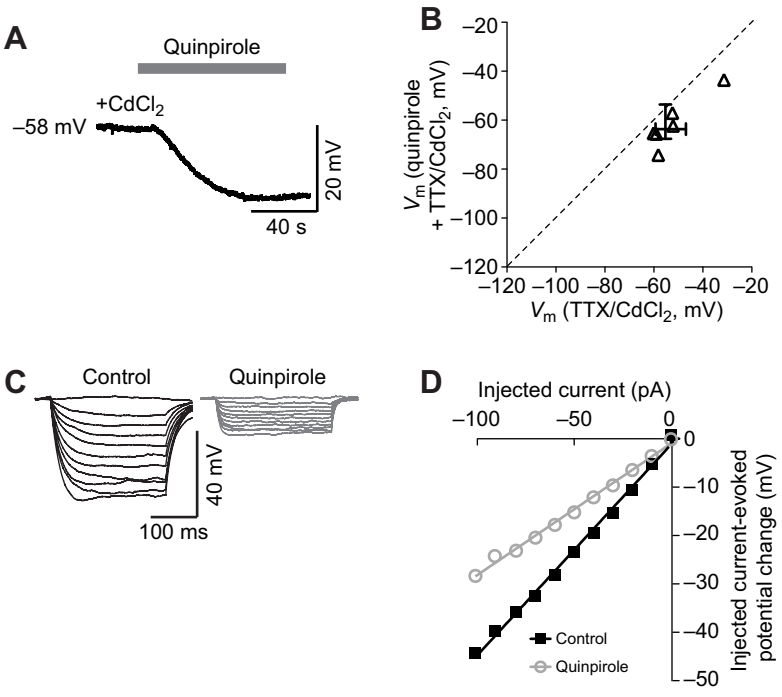

Fig. 3. Mechanisms underlying D2R-mediated hyperpolarization of GnRH1 neurons. (A) Representative intracellular recording from a GnRH1 neuron. Quinpirole (gray bar)-induced membrane hyperpolarization is not abolished by pre-treatment with the synaptic transmission blocker $CdCl_2$. (B) Across the population of recorded neurons, synaptic blockers ($CdCl_2$ and tetrodotoxin, TTX) did not affect membrane hyperpolarization elicited by quinpirole application (N=6 neurons, P=0.03, two-tailed Wilcoxon t-test). Neurons were recorded from brain slices collected from four fish. The cross-hair represents the median effect (center), with IQR. (C) Example plot showing membrane potential changes induced by current injection in a GnRH1 neuron before (left) and after (right) activation of D2Rs via quinpirole bath application. Traces represent sequential injection of current steps, from −100 to 0 pA. (D) Summary of the effect of quinpirole on the voltage–current (V–I) relationship in GnRH1 neurons. Symbols represent the mean current-evoked change in membrane potential. Lines represent linear regressions of the plotted population averages (N=14 neurons, P<0.001, ANCOVA). Each neuron was recorded from a unique brain slice, collected from 11 fish.

D2R-mediated inhibition of GnRH1 neurons is not sexually dimorphic

Previous studies demonstrated sexually dimorphic regulation of dopaminergic signaling in the hypothalamus and pituitary of both mammals and fish (Goebrecht et al., 2014; Saha et al., 2015; Scott et al., 2015). Although androgenic steroids regulate dopamine receptor expression in the fish POA (Pasqualini et al., 2009), there are, to our knowledge, no studies testing for sex-specific differences in dopamine receptor-mediated inhibition of POA neural activity. We further analyzed the data shown in Fig. 2 to test whether the magnitude of dopaminergic inhibition of GnRH1 neurons was sexually dimorphic. Quinpirole application hyperpolarized GnRH1 neurons by an average of −17.8 mV in females (Fig. 2D, red circles; control: −51.8 mV, IQR −61.6 to −47.6 mV; quinpirole: −69.6 mV, IQR −76.0 to −63.4 mV; N=8) and −14.8 mV in males (Fig. 2F, blue triangles; control: −56.7 mV, IQR −60.1 to −47.7 mV; quinpirole: −71.5 mV, IQR −75.7 to −53.7 mV; N=8). The effect of quinpirole on GnRH1 neurons in males was not different from that in females (P=0.70, $F_{1,14}$=0.15, two-way RM ANOVA). These results show that the inhibitory mechanisms postsynaptic to dopamine release onto POA GnRH1 neurons are not sexually dimorphic. However, presynaptic dopamine production or release could vary by sex; sexually dimorphic TH expression, as has been observed in fish and mammals (Saha et al., 2015; Scott et al., 2015), could result in sexually dimorphic regulation of dopaminergic control of reproductive behaviors (Goebrecht et al., 2014).

D2R agonists directly inhibit GnRH1 neurons

Together, the previous experiments suggest that dopaminergic control of GnRH1 release involves D2Rs localized within the *A. burtoni* hypothalamus. The observed effects, however, could result from either dopaminergic activation of inhibitory interneurons, or direct D2R-mediated hyperpolarization of GnRH1 neurons. To distinguish these two mechanisms, we applied quinpirole to GnRH1 neurons while pharmacologically blocking neurotransmission. If the D2R-mediated hyperpolarization requires inhibitory interneurons, blocking synaptic activity will abolish the effect of quinpirole on GnRH1 neurons. Following pre-application of TTX or $CdCl_2$, quinpirole continued to hyperpolarize GnRH1 neurons (Fig. 3A,B; TTX/$CdCl_2$: −55.3 mV, IQR −59.3 to −47.0 mV; quinpirole+TTX/$CdCl_2$: −63.9 mV, IQR −67.8 to −53.8 mV; N=6; P=0.03, Wilcoxon t-test). This hyperpolarization effect was statistically indistinguishable from TTX/$CdCl_2$-free experiments ($F_{1,20}$=2.21, P=0.15, two-way RM ANOVA), indicating that dopaminergic inhibition of GnRH1 neurons is not dependent on inhibitory interneurons but arises, at least in part, from direct interactions between D2Rs and GnRH1 neurons.

Classically, D2Rs produce neuronal inhibition via potassium channel activation (Einhorn et al., 1991). To test whether quinpirole-induced hyperpolarization was due to membrane channel openings, we tested the voltage–current (V–I) relationship of GnRH1 neurons before and after quinpirole application. If quinpirole-induced hyperpolarization results from membrane channels opening, injecting current into treated cells will induce smaller voltage shifts, as leakier membranes are less electrically resistant. Following quinpirole application, sequential current injection steps yielded smaller shifts in GnRH1 neuron membrane voltage, indicative of a dramatic decrease in membrane input resistance (Fig. 3C). We used linear regressions to calculate the slope of the population V–I relationship before and after quinpirole application. The slope of the quinpirole V–I relationship was shallower than the slope of the control V–I relationship (Fig. 3D; control slope: 0.44±0.03 mV pA^{-1}; quinpirole slope: 0.27±0.02 mV pA^{-1}; N=14; $F_{1,304}$=18.70, P<0.001, ANCOVA). Thus, across the population, quinpirole made GnRH1 neuronal membranes less responsive to current injection. Reduced input resistance accompanied by membrane hyperpolarization is consistent with increased conductance through potassium channels (Einhorn et al., 1991). Together, these results strongly suggest that dopaminergic inhibition of GnRH1 neurons is achieved via D2R-mediated opening of potassium channels. Similarly, dopamine regulates hypothalamic GnRH neurons in mice via both D1Rs and D2Rs, the latter also via potassium channels (Liu and Herbison, 2013). The identity of the potassium channel involved in D2R-mediated inhibition of mammalian and fish GnRH neurons is unknown, although candidates include G-protein-coupled inwardly rectifying and ATP-sensitive channels. These channels both control mammalian GnRH neurons via modulation by multiple peptides (Constantin and Wray, 2016; Rønnekleiv and Kelly, 2013; Zhang et al., 2007), and are linked to D2R modulation in other brain regions (Neusch et al., 2000; Perez et al., 2006; Werner et al., 1996).

Using transgenically labeled and *post hoc* identified GnRH1 neurons, we demonstrated that dopamine, acting via D2Rs putatively coupled to potassium channels, inhibits POA GnRH1 neurons. Combined with previous reports, our results demonstrate that dopamine controls reproduction at multiple sites along the teleost HPG axis, and that mechanisms underlying dopaminergic regulation of reproduction are evolutionarily conserved across vertebrates. GnRH1 neurons are master regulators of pituitary activation, and dopaminergic suppression of their activity will profoundly influence the release of pituitary hormones, and thus reproductive behavior. Dopaminergic inhibition of GnRH is important for long-term suppression of reproduction, as occurs before puberty (Becú-Villalobos and Libertun, 1995; Gerber et al., 1984; Lamberts and Wuttke, 1981; Terasawa and Fernandez, 2001), during seasonal anoestrus (Lehman et al., 1997) and as a function of social influences (Davis and Fernald, 1990; Darney et al., 1992; Greenwood, 2003). We propose that the presence of multiple, conserved dopaminergic control sites along the HPG axis emphasizes a privileged role for dopamine in regulating vertebrate reproductive behaviors.

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Competing interests

The authors declare no competing or financial interests.

Author contributions

A.S.B., A.K.G., S.A.J. and R.D.F. designed research; A.S.B. and A.K.G. performed electrophysiology; A.E.B. and S.A.J. performed immunohistochemistry; A.S.B. analyzed the data; A.S.B., S.A.J., A.E.B. and R.D.F. wrote the paper.

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Supplementary information

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References

Becú-Villalobos, D. and Libertun, C. (1995). Development of gonadotropin-releasing hormone (GnRH) neuron regulation in the female rat. *Cell. Mol. Neurobiol.* **15**, 165-176.

Ching, M. and Lin, Y. C. (1994). Ethanol acutely reduces LH and prolactin secretion: possible involvement by dopamine. *Alcohol* **11**, 105-112.
Constantin, S. and Wray, S. (2016). Galanin activates G protein gated inwardly rectifying potassium channels and suppresses Kisspeptin-10 activation of GnRH neurons. *Endocrinology* **157**, 3197-3212.
Contijoch, A. M., Gonzalez, C., Singh, H. N., Malamed, S., Troncoso, S. and Advis, J.-P. (1992). Dopaminergic regulation of luteinizing hormone-releasing hormone release at the median eminence level: immunocytochemical and physiological evidence in hens. *Neuroendocrinology* **55**, 290-300.
Corio, M., Thibault, J. and Peute, J. (1990). Topographical relationships between catecholamine-and neuropeptide-containing fibers in the median eminence of the newt, *Triturus alpestris*. *Cell Tissue Res.* **259**, 561-566.
Darney, K. J., Jr, Goldman, J. M. and Vandenbergh, J. G. (1992). Neuroendocrine responses to social regulation of puberty in the female house mouse. *Neuroendocrinology* **55**, 434-443.
Davis, M. R. and Fernald, R. D. (1990). Social control of neuronal soma size. *J. Neurobiol.* **21**, 1180-1188.
Dufour, S., Weltzien, F.-A., Sebert, M.-E., Le Belle, N., Vidal, B., Vernier, P. and Pasqualini, C. (2005). Dopaminergic inhibition of reproduction in teleost fishes: ecophysiological and evolutionary implications. *Ann. N. Y. Acad. Sci.* **1040**, 9-21.
Dufour, S., Sebert, M.-E., Weltzien, F.-A., Rousseau, K. and Pasqualini, C. (2010). Neuroendocrine control by dopamine of teleost reproduction. *J. Fish Biol.* **76**, 129-160.
Einhorn, L. C., Gregerson, K. A. and Oxford, G. S. (1991). D2 dopamine receptor activation of potassium channels in identified rat lactotrophs: whole-cell and single-channel recording. *J. Neurosci.* **11**, 3727-3737.
Fernald, R. D. and Hirata, N. R. (1977). Field study of *Haplochromis burtoni*: quantitative behavioural observations. *Anim. Behav.* **25**, 964-975.
Gerber, P., Döcke, F., Rohde, W. and Dörner, G. (1984). Evidence that inhibition of medial preoptic dopaminergic activity may be involved in the prepubertal desensitization to the negative oestrogen feedback in female rats. *Exp. Clin. Endocrinol.* **84**, 7-12.
Goebrecht, G. K. E., Kowtoniuk, R. A., Kelly, B. G. and Kittelberger, J. M. (2014). Sexually-dimorphic expression of tyrosine hydroxylase immunoreactivity in the brain of a vocal teleost fish (*Porichthys notatus*). *J. Chem. Neuroanat.* **56**, 13-34.
Greenwood, A. K. (2003). Plasticity in the neural control of reproductive behavior and physiology. *PhD Thesis*, Stanford University.
Greenwood, A. K. and Fernald, R. D. (2004). Social regulation of the electrical properties of gonadotropin-releasing hormone neurons in a cichlid fish (Astatotilapia burtoni). *Biol. Reprod.* **71**, 909-918.
Jennes, L., Stumpf, W. E. and Tappaz, M. L. (1983). Anatomical relationships of dopaminergic and GABAergic systems with the GnRH-systems in the septo-hypothalamic area. Immunohistochemical studies. *Exp. Brain Res.* **50**, 91-99.
Kuljis, R. O. and Advis, J. P. (1989). Immunocytochemical and physiological evidence of a synapse between dopamine- and Luteinizing Hormone Releasing Hormone-containing neurons in the ewe median eminence. *Endocrinology* **124**, 1579-1581.
Lacau-Mengido, I. M., Becú-Villalobos, D., Thyssen, S. M., Rey, E. B., Lux-Lantos, V. A. R. and Libertun, C. (1993). Antidopaminergic-Induced hypothalamic LHRH release and pituitary gonadotrophin secretion in 12 day-old female and male rats. *J. Neuroendocrinol.* **5**, 705-709.
Lamberts, R. and Wuttke, W. (1981). Puberty of female rats may in part be explained by decreased hypothalamic dopamine receptor sensitivity. *Brain Res.* **215**, 375-381.
Lehman, M. N., Karsch, F. J. and Silverman, A.-J. (1988). Potential sites of interaction between catecholamines and LHRH in the sheep brain. *Brain Res. Bull.* **20**, 49-58.
Lehman, M. N., Goodman, R. L., Karsch, F. J., Jackson, G. L., Berriman, S. J. and Jansen, H. T. (1997). The GnRH system of seasonal breeders: anatomy and plasticity. *Brain Res. Bull.* **44**, 445-457.
Leranth, C., MacLusky, N. J., Shanabrough, M. and Naftolin, F. (1988). Catecholaminergic innervation of luteinizing hormone-releasing hormone and glutamic acid decarboxylase immunopositive neurons in the rat medial preoptic area. An electron-microscopic double immunostaining and degeneration study. *Neuroendocrinology* **48**, 591-602.
Levavi-Sivan, B., Ofir, M. and Yaron, Z. (1995). Possible sites of dopaminergic inhibition of gonadotropin release from the pituitary of a teleost fish, tilapia. *Mol. Cell. Endocrinol.* **109**, 87-95.
Liu, X. and Herbison, A. E. (2013). Dopamine regulation of gonadotropin-releasing hormone neuron excitability in male and female mice. *Endocrinology* **154**, 340-350.
Liu, Y., Wang, L., Liu, J. and Di, Y. (2013). A study of human skin and surface temperatures in stable and unstable thermal environments. *J. Therm. Biol.* **38**, 440-448.
Ma, Y., Juntti, S. A., Hu, C. K., Huguenard, J. R. and Fernald, R. D. (2015). Electrical synapses connect a network of gonadotropin releasing hormone neurons in a cichlid fish. *Proc. Natl. Acad. Sci. USA* **112**, 3805-3810.
Miller, S. M. and Lonstein, J. S. (2009). Dopaminergic projections to the medial preoptic area of postpartum rats. *Neuroscience* **159**, 1384-1396.
Neusch, C., Runde, D. and Moser, A. (2000). G proteins modulate D2 receptor-coupled K(ATP) channels in rat dopaminergic terminals. *Neurochem. Res.* **25**, 1521-1526.
O'Connell, L. A., Fontenot, M. R. and Hofmann, H. A. (2010). Characterization of the dopaminergic system in the brain of an African cichlid fish, *Astatotilapia burtoni*. *J. Comp. Neurol.* **519**, 75-92.
Owens, R. E., Fleeger, J. L. and Harms, P. G. (1980). Evidence for central nervous system (CNS) involvement in inhibition of luteinizing hormone (LH) release by dopamine receptor stimulation. *Endocr. Res. Commun.* **7**, 99-105.
Pasqualini, C., Weltzien, F.-A., Vidal, B., Baloche, S., Rouget, C., Gilles, N., Servent, D., Vernier, P. and Dufour, S. (2009). Two distinct dopamine D2 receptor genes in the European eel: molecular characterization, tissue-specific transcription, and regulation by sex steroids. *Endocrinology* **150**, 1377-1392.
Pehrson, J. J., Jaffee, W. L. and Vaitukaitis, J. L. (1983). Effect of dopamine on gonadotropin-releasing hormone-induced gonadotropin secretion in postmenopausal women. *J. Clin. Endocrinol. Metab.* **56**, 889-892.
Perez, M. F., White, F. J. and Hu, X.-T. (2006). Dopamine D(2) receptor modulation of K(+) channel activity regulates excitability of nucleus accumbens neurons at different membrane potentials. *J. Neurophysiol.* **96**, 2217-2228.
Pompolo, S., Pereira, A., Kaneko, T. and Clarke, I. J. (2003). Seasonal changes in the inputs to gonadotropin-releasing hormone neurones in the ewe brain: an assessment by conventional fluorescence and confocal microscopy. *J. Neuroendocrinol.* **15**, 538-545.
Rønnekleiv, O. K. and Kelly, M. J. (2013). Kisspeptin excitation of GnRH neurons. *Adv. Exp. Med. Biol.* **784**, 113-131.
Saha, S., Patil, S., Singh, U., Singh, O. and Singru, P. S. (2015). Sexual dimorphism in the hypophysiotropic tyrosine hydroxylase-positive neurons in the preoptic area of the teleost, *Clarias batrachus*. *Biol. Sex Differ.* **6**, 23.
Scott, N., Prigge, M., Yizhar, O. and Kimchi, T. (2015). A sexually dimorphic hypothalamic circuit controls maternal care and oxytocin secretion. *Nature* **525**, 519-522.
Tasaka, K., Miyake, A., Sakumoto, T. and Aono, T. (1985). Dopamine decreases release of luteinizing hormone releasing hormone from superfused rat mediobasal hypothalamus. *J. Endocrinol. Invest.* **8**, 373-376.
Terasawa, E. and Fernandez, D. L. (2001). Neurobiological mechanisms of the onset of puberty in primates. *Endocr. Rev.* **22**, 111-151.
Tillet, Y., Caldani, M. and Batailler, M. (1989). Anatomical relationships of monoaminergic and neuropeptide Y-containing fibres with luteinizing hormone-releasing hormone systems in the preoptic area of the sheep brain: immunohistochemical studies. *J. Chem. Neuroanat.* **2**, 319-326.
Ugrumov, M., Hisano, S. and Daikoku, S. (1989). Topographic relations between tyrosine hydroxylase- and luteinizing hormone-releasing hormone-immunoreactive fibers in the median eminence of adult rats. *Neurosci. Lett.* **102**, 159-164.
Vacher, C., Ferrière, F., Marmignon, M.-H., Pellegrini, E. and Saligaut, C. (2002). Dopamine D2 receptors and secretion of FSH and LH: role of sexual steroids on the pituitary of the female rainbow trout. *Gen. Comp. Endocrinol.* **127**, 198-206.
Van Goor, F., Goldberg, J. I. and Chang, J. P. (1998). Dopamine-D2 actions on voltage-dependent calcium current and gonadotropin-II secretion in cultured goldfish gonadotrophs. *J. Neuroendocrinol.* **10**, 175-186.
Werner, P., Hussy, N., Buell, G., Jones, K. A. and North, R. A. (1996). D2, D3, and D4 dopamine receptors couple to G protein-regulated potassium channels in Xenopus oocytes. *Mol. Pharmacol.* **49**, 656-661.
White, S. A., Kasten, T. L., Bond, C. T., Adelman, J. P. and Fernald, R. D. (1995). Three gonadotropin-releasing hormone genes in one organism suggest novel roles for an ancient peptide. *Proc. Natl. Acad. Sci. USA* **92**, 8363-8367.
Yu, K. L. and Peter, R. E. (1992). Adrenergic and dopaminergic regulation of gonadotropin-releasing hormone release from goldfish preoptic-anterior hypothalamus and pituitary in vitro. *Gen. Comp. Endocrinol.* **85**, 138-146.
Yu, K. L., Rosenblum, P. M. and Peter, R. E. (1991). In vitro release of gonadotropin-releasing hormone from the brain preoptic-anterior hypothalamic region and pituitary of female goldfish. *Gen. Comp. Endocrinol.* **81**, 256-267.
Zhang, C., Bosch, M. A., Levine, J. E., Rønnekleiv, O. K. and Kelly, M. J. (2007). Gonadotropin-releasing hormone neurons express KATP channels that are regulated by estrogen and responsive to glucose and metabolic inhibition. *J. Neurosci.* **27**, 10153-10164.