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 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 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...
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% O2, 5% CO2) slicing solution containing (in mmol l−1): 234 sucrose, 11 glucose, 24 NaHCO3, 2.5 KCl, 1.25 NaHPO4, 10 MgSO4 and 0.5 CaCl2, embedded in 4% low melting 1): 234 sucrose, 11 glucose, 24 NaHCO3, 2.5 KCl, 1.25 NaHPO4, 10 MgSO4 and 0.5 CaCl2, embedded in 4% low melting

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 Vrest: −51.3 mV, IQR −55.4 to −48.0 mV; dopamine Vrest: −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.
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. 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=8 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=0.03, two-tailed Wilcoxon t-test). Each neuron was recorded from a unique brain slice, collected from eight fish. Conventions as for B.

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 pretreatment with the synaptic transmission blocker CdCl₂. (B) Across the population of recorded neurons, synaptic blockers (CdCl₂ 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, F1,14=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 CdCl2, quinpirole continued to hyperpolarize GnRH1 neurons (Fig. 3A,B; TTX/CdCl2: −55.3 mV, IQR −59.3 to −47.0 mV; quinpirole+TTX/CdCl2: −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/CdCl2–free experiments (F1,20=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; F1,304=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 GnRH1 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; Ronneklev 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 GnRH1 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


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Supplementary information

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References


