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Prostaglandin E₂ release from astrocytes triggers gonadotropin-releasing hormone (GnRH) neuron firing via EP2 receptor activation

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Abstract

Astrocytes in the hypothalamus release prostaglandin E₂ (PGE₂) in response to cell-cell signaling initiated by neurons and glial cells. Upon release, PGE₂ stimulates the secretion of gonadotropin-releasing hormone (GnRH), the neuropeptide that controls reproduction, from hypothalamic neuroendocrine neurons. Whether this effect on GnRH secretion is accompanied by changes in the firing behavior of these neurons is unknown. Using patch-clamp recording we demonstrate that PGE₂ exerts a dose-dependent postsynaptic excitatory effect on GnRH neurons. These effects are mimicked by an EP2 receptor agonist and attenuated by protein kinase A inhibitors. The acute blockade of prostaglandin synthesis by indomethacin or the selective inhibition of astrocyte metabolism by fluoroacetate suppresses the spontaneous firing activity of GnRH neurons in brain slices. Similarly, GnRH neuronal activity is reduced in mice with impaired astrocytic PGE₂ release due to defective erbB signalling in astrocytes. These results indicate that astrocyte-to-neuron communication in the hypothalamus is essential for the activity of GnRH neurons and suggest that PGE₂ acts as a gliotransmitter within the GnRH neurosecretory system.

Key words: LHRH, glia-to-neuron signaling, gliotransmission, cyclooxygenase, hypothalamus

\body

Introduction

It is increasingly clear that astrocytes play an important role in maintaining central nervous system function (1-3) and controlling key bodily processes, such as breathing (4), sleep (5), and reproduction (6). Because of their perivascular and interneuronal localization, astrocytes are well positioned to sense afferent neuronal and blood-borne signals, and ideally suited for the temporal and spatial propagation of these signals (7-9). The activation of astrocytes leads to the release of gliotransmitters (8, 10) that trigger rapid responses in neighboring cells and thus contribute to the region-specific homeostatic regulation of neuronal function.

In the hypothalamus, astrocytes regulate the secretory activity of neuroendocrine neurons (11-14). A subset of such neurons secretes the decapeptide gonadotropin hormone-releasing hormone (GnRH), which controls both the initiation of puberty and adult reproductive function. In rodents, GnRH neurons are mostly located in the preoptic region of the ventral forebrain. They project to the median eminence of the hypothalamus, where GnRH is released into the pituitary portal blood for delivery to the anterior pituitary. In the pituitary, GnRH elicits the secretion of luteinizing hormone and follicle-stimulating hormone, which stimulate gametogenesis and gonadal hormone secretion and thus support reproductive function. It is now clear that the secretory activity of GnRH neurons is controlled by both neuronal and glial input (12, 13, 15, 16). While glutamate is a key neurotransmitter involved in the trans-synaptic activation of GnRH neurons (17, 18), prostaglandin E₂ (PGE₂) mediates cell-cell communication between astrocytes and GnRH neurons (6, 19, 20). A functional connection between the two systems is demonstrated by the ability of glutamate to elicit PGE₂ release from astrocytes (21-23). In the hypothalamus, the glutamate-dependent activation of PGE₂ release involves astroglial erbB signaling (22), a crucial component of the cell-cell communication process used by astrocytes to facilitate neuroendocrine reproductive development and adult function (6, 24). Whether PGE₂ affects the electrical activity of GnRH neurons is, however, unknown. Here we report a remarkably potent postsynaptic excitatory

effect of PGE₂ on GnRH neurons that requires the activation of the EP2 subclass of PGE₂ receptors and of cAMP/PKA-mediated downstream signaling pathways.

Results

PGE₂ activates adult GnRH neurons in a potent and reversible manner

We used whole-cell patch-clamp recording to study the electrical activity of 193 GnRH neurons in brain slices containing the preoptic region from 175 adult transgenic mice (61 males and 114 females) expressing green fluorescent protein (GFP) under the control of the GnRH promoter (25). The pipette solution used (ps1) resulted in an average resting potential of -75.54 ± 0.38 mV (n=151) and an input resistance of 1417.76 ± 43.07 M Ω (n=97). In this configuration, 87% of the neurons remained silent at the resting potential (Supplementary results and Suppl. Fig. 1). Bath application of PGE₂ resulted in a striking, dose-dependent depolarizing effect on GnRH neurons (Fig. 1A-C), with an EC₅₀ of 0.018 μ M (Fig. 1C,D). Among all the GnRH neurons treated with 1 μ M PGE₂, 78% (91 out of 116) depolarized rapidly (within 10 to 190 s; mean 41.00 ± 3.21 s) (Fig. 1A-C). PGE₂-induced (1 μ M) depolarization had a mean amplitude of 8.95 ± 0.32 mV (n=91) and was accompanied by the sustained generation of action potentials (Fig. 1A-C) and a decrease in membrane resistance (Fig. 1C). Interestingly, 33 cells out of 91 responded to PGE₂ with bursting activity (Fig. 1B) similar to that which occurred spontaneously in some neurons (Suppl. Fig. 1B,C) and which is thought to be essential for neuropeptide secretion from neuroendocrine cells (26). The PGE₂ excitatory effect was fully reversible in 73 neurons (Fig.1A-C), whereas 18 neurons did not recover their basal membrane potential after cessation of the PGE₂ treatment. No difference in the response of GnRH neurons to PGE₂ was detected between animals of different sex or stages of the estrous cycle (Suppl. Table 1). Because PGE₂ has previously been shown to stimulate glutamate release from astrocytes (21), we next examined the effects of PGE₂ on GnRH-GFP neurons in the presence of 20 μ M CNQX and 100 μ M DL-AP5 (see methods), to block AMPA/kainate and NMDA receptors, respectively. Under these conditions, the membrane depolarization induced by 1 μ M PGE₂ was not altered ($10.62 \pm$

1.20 mV, n=4; Fig. 1E), indicating that glutamate is not an intermediary in the excitatory effects of PGE₂ on GnRH neuronal activity.

PGE₂ maintained its potent stimulatory effect on the firing behavior of GnRH neurons recorded using the loose patch-clamp configuration (Fig. 1F). This indicates that the dilution of the intracellular compartment by the patch electrode medium in the whole-cell configuration does not alter the response of GnRH neurons to PGE₂. In the loose patch-clamp configuration, GnRH neurons were spontaneously active and all responded to the addition of 1 μM PGE₂ to the perfusion medium by a transient acceleration of the action potential discharge (0.51±0.15 Hz before treatment vs. 1.35±0.12 Hz with bath application of PGE₂, *t* test; *p* < 0.05; n=5; Fig. 1F). To investigate whether PGE₂ had similarly consistent excitatory effects on the electrical activity of non-GnRH neurons in the preoptic region, 14 non-GFP-labeled neurons located near GnRH-GFP neurons were recorded. While 10 neurons responded to the bath application of 1 μM PGE₂ with either an acceleration (n=6) or an inhibition (n=4, Fig. 1G) of their firing pattern, four were insensitive to the prostaglandin.

PGE₂ elicits membrane depolarization of GnRH neurons via a postsynaptic effect involving activation of an inward current

The effect of PGE₂ was further investigated in the presence of tetrodotoxin (TTX; 0.5-1 μM) to block action potential-dependent synaptic transmission, in addition to the glutamate and GABA_A receptor antagonists DL-AP5 (100 μM), CNQX (20 μM) and bicuculline (20 μM) to block ionotropic receptor mediated presynaptic inputs. Under these conditions, PGE₂ (1 μM) consistently depolarized the membrane potential of GnRH neurons (Fig. 2A) with an average depolarization of 7.72 ± 0.48 mV (n=10). The current-voltage relationship before and after applying PGE₂ (1 μM) was obtained by injecting a series of square wave currents from -70 to +70 pA (Fig. 2B1,B2). The input resistance was calculated based on the linear part of the current-voltage curve (Fig. 2B3). PGE₂ (1 μM) decreased the input resistance by 27.21 ± 2.38% (n=10), indicating an increase in conductance. These results suggest that PGE₂ triggers firing in GnRH neurons via a direct postsynaptic mechanism.

To identify the conductance involved in the PGE₂-induced membrane depolarization, we performed voltage-clamp recordings at a holding potential of -70 mV, and observed that 1 μM PGE₂ elicited an inward current of 22.92 ± 5.57 pA (n=8) in the presence of TTX (0.5 μM), DL-AP5 (100 μM), CNQX (20 μM), and bicuculline (20 μM). This current appeared 40.00 ± 13.89 s (10 to 130 s, n=8) after the initiation of PGE₂-treatment and ended 193.75 ± 32.07 s (n=8) after the removal of PGE₂ from the bath solution (Fig. 2C). We then investigated the current evoked by PGE₂ using a ramp voltage-clamp protocol from -120 mV to -30 mV and a holding potential of -70 mV, in the presence of TTX (0.5 μM), DL-AP5 (100 μM), CNQX (20 μM), and bicuculline (20 μM; Fig. 2D1). PGE₂ (1 μM) increased the current evoked by this protocol (Fig. 2D1). After subtracting control values from the current recorded after applying PGE₂, a PGE₂-induced inward current was obtained (Fig. 2D2). This inward current had a linear voltage dependence from -120 to -40 mV and was suppressed at a mean value of -41.12 ± 3.54 mV (n=4; Fig. 2D2), suggesting the activation of a non-selective cation conductance (27, 28). This value was also close to the membrane potential measured in current-clamp mode (-45.66 ± 1.68 mV, *t* test; *P* > 0.05; n=7; Fig. 2B3), for which the two current-voltage curves obtained in the presence and absence of PGE₂ converged.

EP2 receptors are expressed in GnRH neurons and their activation mimics the effects of PGE₂

To gain insight into the nature of the receptors that mediate the potent postsynaptic excitatory action of PGE₂ on GnRH neurons, we tested the effects of 17-phenyl trinor PGE₂ (17PT-PGE₂), sulprostone and butaprost, which are EP1, EP1/EP3 and EP2 receptor agonists (29), respectively, on GnRH neuronal activity. The bath application of 17PT-PGE₂ or sulprostone at 10 μM, a concentration previously shown to promote GnRH release in GnRH-secreting cell lines (20), had no effect on GnRH neuronal activity (Fig. 3A,B). However, 10 μM butaprost resulted in membrane depolarization (Fig. 3A,B). Analysis of the voltage-clamp ramps in the presence or absence of butaprost (Fig. 3C1) revealed that this EP2 receptor agonist activated an inward current that was suppressed at a mean value of -48.33 ± 6.23 mV

(n=3, Fig. 3C2), close to the value obtained with PGE₂ (Fig. 2D2; *t* test, *P* > 0.05). Perfusion of the slices with 30 μM AH 23848, an antagonist of the EP4 receptor (29), did not modify the response of GnRH neurons to PGE₂ treatment (n = 3). Taken together, these observations strongly suggest that the excitatory effects of PGE₂ on GnRH neuronal activity are mediated by EP2 receptor activation. To confirm the presence of EP2 receptors in GnRH neurons, we used immunohistochemistry. The EP2 receptor was abundantly expressed in the preoptic region; among the 128 GnRH-GFP neurons analyzed, 72 (56 %) displayed EP2 receptor immunostaining (n = 4 animals) (Fig. 3D).

PGE₂-mediated membrane depolarization in GnRH neurons requires protein kinase A activation

Since EP2 receptors are linked to the Gs-cAMP-protein kinase A (PKA) pathway (29, 30), we used PKA inhibitors to determine whether the excitatory effect of PGE₂ on GnRH neurons could be inhibited or attenuated. In the presence of 0.5 μM TTX, the bath application of the PKA inhibitors H89 (10 μM, n=5) and KT 5720 (10 μM, n=3), or the competitive PKA antagonist Rp-cAMP (20 μM, n=3) for 30 min significantly attenuated the stimulatory effect of PGE₂ on membrane depolarization in GnRH neurons (Fig. 3E,F). The activation of EP1 and EP3 receptors is coupled to the mobilization of intracellular calcium stores (29), but did not cause membrane depolarization in GnRH neurons (Fig. 3A,B). Consistent with this observation, the depletion of intracellular calcium stores with thapsigargin (2 μM) did not block the depolarizing effects of PGE₂ on GnRH neurons (n = 2). Taken together, our data suggest that the excitatory effects of PGE₂ on GnRH neuronal activity are exerted via an EP2-Gs-cAMP/PKA signaling pathway.

Blockade of endogenous cyclooxygenase activity inhibits spontaneous firing of GnRH neurons

To monitor spontaneous GnRH neuronal activity, whole-cell patch-clamp recordings were performed using a pipette solution (ps2) that conferred the cells with an average resting

potential of -61.50 ± 0.62 mV ($n=10$) and an input resistance of 1384.27 ± 73.71 M Ω ($n=10$), as shown previously (31). At this resting potential, all neurons exhibited spontaneous activity with a mean discharge of 0.45 ± 0.07 Hz ($n=10$). To explore the contribution of PGE₂ to this spontaneous activity, we bath-applied indomethacin, an inhibitor of cyclooxygenase, the rate-limiting enzyme in prostaglandin synthesis, to slices of the preoptic region during recording from GnRH neurons (Fig. 4). Bath application of this inhibitor at 50-100 μ M either greatly reduced (by 95%; $n=3$; Fig. 4A) or fully suppressed ($n=5$; Fig. 4B) the spontaneous discharge of GnRH neurons (Fig. 4C). These effects were either reversible ($n=2$) or irreversible ($n=6$). Lower concentrations of indomethacin (5-10 μ M) had no effect on GnRH neuronal activity, except in the case of one cell that exhibited a reversible reduction of its basal firing rate by 57%. At 100 μ M indomethacin, the suppression of firing was accompanied by a membrane hyperpolarization (4.5 ± 0.6 mV, $n = 6$) (Fig. 4B). However, the bath application of PGE₂ (1 μ M) remained capable of triggering membrane depolarization (8.12 ± 2.39 mV) as well as action potentials ($n=4$) (Fig. 4B). These experiments suggest that the endogenous production of PGE₂ contributes to the maintenance of the activity of GnRH neurons in the preoptic region.

Astrocytic PGE₂ regulates GnRH neuronal activity *in situ*

Hypothalamic astrocytes are a source of PGE₂ (19), which is required for the normal release of GnRH (6, 24). To determine whether astrocytes contribute to the regulation of GnRH neuronal firing, we pre-treated hypothalamic slices with the glial toxin fluoroacetate (5 mM; 60-120 min) (2, 32-34). Fluoroacetate significantly impaired astrocyte function as assessed by the inability of astrocytes to take up sulforhodamine 101 (SR101) (Fig. 4D), a fluorescent dye that is selectively taken up by astrocytes both *in vivo* (35) and in living brain slices (36). The number of astrocytes capable of taking up the dye in the vicinity of GnRH neurons was reduced by $78 \pm 1\%$ when compared to untreated slices ($n=4$; t-test, $p < 0.01$). The spontaneous firing of GnRH neurons (detected using ps2 conditions) was strikingly reduced in fluoroacetate-treated slices (9 out of 12) (Fig. 4C,E). The remaining neurons (3 out of 12)

either exhibited a sporadic pattern of action potential discharge (0.01 Hz, n=2), or fired in repetitive bursts (0.15 Hz, n=1). In contrast, fluoroacetate treatment did not affect the basal membrane properties of GnRH neurons (resting potential of -74.29 ± 1.44 mV and input resistance of 1123.50 ± 130.18 M Ω in ps1, n=7; t-test for treated vs. control slices; $p > 0.05$). Importantly, in most GnRH neurons, fluoroacetate treatment did not affect the membrane depolarizing effect of PGE₂ (1 μ M; 6 out of 9 neurons; 7.92 ± 0.42 mV; Fig. 4E). The treatment also failed to affect the latency of the membrane depolarization upon initiation of PGE₂ treatment (50.00 ± 13.36 s in ACSF vs. 31.25 ± 6.10 s in FA, n = 8 for each treatment, t-test, $p > 0.05$), the duration of the effect (234.28 ± 36.70 s in ACSF vs. 377.14 ± 74.08 s in FA, n = 7 for each treatment, t-test, $p > 0.05$) or the ability of the prostaglandin to trigger action potentials (5 out of 6 neurons, Suppl. Fig. 2).

We next sought to investigate the firing activity of GnRH neurons in mice with deficient astrocytic PGE₂ production. We used transgenic mice expressing a dominant-negative form of the erbB4 receptor (DN-erbB4) specifically targeted to astrocytes by means of the human GFAP promoter (6). This mutated receptor blocks the ligand-dependent activation of erbB2 and erbB4 receptors without affecting signaling through other receptors, such as erbB1 or Notch1 (6, 37). GnRH secretion is deficient in GFAP-DN-erbB4 mice due to the inability of astrocytes to respond to erbB4 activation by producing PGE₂ (6). To visualize GnRH neurons in these animals we crossed GFAP-DN-erbB4 mice with GnRH-GFP animals. GnRH neurons recorded from four double-transgenic mice under ps2 conditions were either silent (n=5; Fig. 4F) or displayed a sporadic low-frequency (0.02 Hz) pattern of action potential discharge (n=1), in contrast to GnRH-GFP neurons recorded from wild-type controls (Fig. 4C). These results suggest that the excitability of GnRH neurons is reduced in GFAP-DN-erbB4 mice due to the failure of PGE₂ production by erbB4-deficient astrocytes. We tested this assumption by determining whether exogenous PGE₂ could restore GnRH membrane depolarization in GFAP-DN-erbB4 mice. The bath application of PGE₂ (1 μ M) elicited membrane depolarization equally strongly in hypothalamic slices from wild-type and GFAP-

DN-erbB4 mice (8.67 ± 1.32 mV in DN-erbB4 mutant mice vs. 8.72 ± 0.67 mV in wild-type controls, $n = 6$ and 10 , respectively, t-test, $p > 0.05$) (Fig. 4F).

Discussion

The disruption of astrocytic erbB4 receptor signaling by the overexpression of a dominant-negative erbB4 receptor leads to diminished astrocytic PGE₂ release in response to ligand-dependent erbB4 activation, leading in turn to reduced GnRH release, delayed puberty and disrupted adult reproductive function (6, 24). The present results show that GnRH neuronal activity is decreased in these animals, and that this deficiency is mimicked by the bath application of either fluoroacetate, an inhibitor of astrocyte metabolism (2, 32, 34), or the cyclooxygenase blocker indomethacin, to slices of the preoptic region from wild-type animals. Our findings that GnRH neurons respond to PGE₂ with an enhancement of the firing rate, and that PGE₂ rescues GnRH neuronal activity in brain slices with reduced astrocytic PGE₂ output indicate that glial PGE₂ is an important component of the homeostatic mechanism controlling GnRH neuronal activity.

The presence of EP2 receptors in GnRH neurons ((38), present study), and the finding that the selective EP2 receptor agonist butaprost mimics the effect of PGE₂, suggest that the excitatory effect of PGE₂ on GnRH neuronal activity involves the activation of EP2 receptors. This inference is supported by an earlier report showing that the stimulation of GnRH-producing GT1-7 cells with butaprost results in GnRH release (16). In addition, the attenuation of the effect of PGE₂ by the bath application of PKA inhibitors or biologically inactive cAMP is consistent with earlier findings demonstrating the coupling of the EP2 receptor to the cAMP/PKA pathway (29) and the involvement of cAMP in the intracellular mechanism underlying the stimulatory effect of PGE₂ on GnRH secretion (39). A previous study has reported that native GnRH neurons also express EP1 receptors (20), which are coupled to the mobilization of intracellular calcium stores (29) and to GnRH release (20). Our electrophysiological data show that the EP1 receptor agonists 17PT-PGE₂ and sulprostone had no effect on GnRH neuronal activity, and that the depletion of intracellular calcium stores

with thapsigargin did not alter the depolarizing response of GnRH neurons to PGE₂. These results, together with earlier findings demonstrating that PGE₂-induced GnRH release from median eminence explants requires the mobilization of intracellular calcium stores (40), suggest that the EP1 receptor-dependent stimulation of GnRH release is exerted at the level of GnRH nerve terminals rather than GnRH cell bodies. Thus, PGE₂ may stimulate both GnRH neuron firing and GnRH release by acting at the cell soma and nerve terminal levels, respectively.

An intriguing finding in this study is that the cyclooxygenase (COX) inhibitor indomethacin failed to affect spontaneous GnRH neuronal activity at doses lower than 50 μM, which is > 1700 times the EC₅₀ of COX-1 (0.028 μM), but only ~ 25 times the EC₅₀ of COX-2 (1.68 μM) (41). This finding suggests that eicosanoids sustaining spontaneous GnRH neuronal activity are mostly COX-2 derived products, a conclusion consistent with evidence that COX-2 is the most abundant COX form expressed in astrocytes (42, 43). Because endocannabinoids, such as 2-arachidonoyl glycerol (2-AG) and arachidonoyl ethanolamide (AEA or anandamide) were recently shown to be substrates for COX-2 (44) and because endocannabinoids modulate GABAergic excitatory inputs to GnRH neurons (45), we cannot rule out that part of the effects of indomethacin on the spontaneous activity of GnRH neurons may be due to inhibition of endocannabinoid oxygenation by COX-2 (46).

Equally intriguing is the apparent lack of sex and estrous cycle effects of PGE₂ on GnRH neuronal activity. Previous studies have shown that GnRH firing activity is modified by estradiol in a diurnal dependent manner (47), and that COX-2 expression in the preoptic region is greater in males than females (48). Perhaps the lack of changes in GnRH firing activity is related to the fact that all recordings were made at the same time of the day and using a saturating dose of PGE₂. In addition to its postsynaptic effect on GnRH neurons, PGE₂ may also modulate the activity of steroid-sensitive afferent neuronal populations (49), including kisspeptin neurons that ensure coordinated progression of neuroendocrine events sustaining ovulatory cyclicity (50). The recent development of a mouse model that enables

identification of kisspeptin neurons using fluorescent reporter genes (51) now renders these neurons amenable to scrutiny.

PGE₂ has long been known to play a role in the control of GnRH neuronal function (52, 53). More recent work points to PGE₂ as a mediator of astrocyte-to-GnRH-neuron communication initiated by the activation of erbB signaling in astrocytes (6, 19, 24, 54). Astrocytes, which are sophisticated sensors of neuronal activity (7, 8), release PGE₂ at active synapses in response to glutamate (21, 23, 55). Interestingly, a ligand-dependent increase in erbB signaling appears to mediate glutamate-stimulated PGE₂ release in hypothalamic astrocytes (22). Within the hypothalamus, both glutamate and glial erbB signaling play physiological roles in promoting GnRH release at the onset of puberty (6, 18, 54, 56) and during adult reproductive life (17, 24). The present results identify PGE₂ as a potent excitatory regulator of GnRH neuronal activity, and indicate that the production of PGE₂ by astroglial cells plays a hitherto unappreciated role in the homeostatic regulation of GnRH neuronal excitability.

Materials and Methods

Animals. Electrophysiological recordings were performed on adult GnRH-GFP and GFAP-DN-erbB4/GnRH-GFP transgenic mice. The generation of GFAP-DN-erbB4 mice has been described previously (6).

Electrophysiological experiments. GnRH-GFP neurons were recorded as described in a previous study (31). Pipette solution 1 (ps1) contained (in mM) K-gluconate, 125; HEPES, 10; CaCl₂, 1; MgCl₂, 1; ATP-Mg, 2; EGTA, 11; GTP, 0.3; NaCl, 15 (pH: 7.3 with KOH, osmolarity: 270-280 mOsm). Pipette solution 2 (ps2) contained (in mM): K-gluconate, 140; HEPES, 10; ATP-Mg, 2; EGTA, 1; KCl, 10 (pH: 7.3 with KOH, osmolarity: 270-290 mOsm). Drugs were applied to the perfusing system (bath application) to obtain the final concentrations indicated. The drugs used were: PGE₂, Butaprost, Sulprostone, 17-phenyl trinor PGE₂ (Cayman chemical); 6-cyano-7-nitroquinoxaline-2,3-dione (CNQX), DL-2-Amino-

5-phosphonopentanoic acid (DL-AP5), Tetrodotoxin citrate (TTX), Thapsigargin (Tocris); Sodium fluoroacetate, Indomethacin, AH 23848, H-89, KT 5720, Rp-cAMPS and Bicuculline methiodide (Sigma). Detailed methods are provided in *SI Materials and Methods*.

Fluorescent staining. Detection of EP2 receptor (rabbit polyclonal antibody, Cayman Chemical) and SR101 uptake were performed using protocols described previously in references (6) and (36), respectively. Detailed methods are provided in *SI Materials and Methods*.

Data analysis. Statistical analysis was performed using Sigma-Stat software (Jandel, San Raphael, CA). Differences between several groups were analyzed by one-way ANOVA, followed by a Student-Newman-Keuls multiple comparison test. The Student's *t* test was used to compare two groups. The data points of the dose-response curve were fitted with a four-parameter logistic curve using Sigma Plot 2001 (SPSS, Chicago, IL). A *p*-value of < 0.05 was considered to indicate a statistically significant difference. Values are reported as the mean \pm the standard error of the mean (S.E.M.).

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Figure legends

Figure 1. PGE₂ powerfully activates GnRH neurons. **A-B**, Whole-cell current-clamp recordings showing the effect of bath application of PGE₂ on two GnRH neurons. Note that in these two silent GnRH neurons, which had a resting membrane potential of -74 mV (**A**) and -68 mV (**B**) respectively, PGE₂ induced a reversible membrane depolarization that led to the initiation of spike firing. The effect was short-lived in **A** and long-lasting in **B**. Note that in **B**, the spike firing started with a bursting pattern (asterisks). **C**, Whole-cell current-clamp recording of a single GnRH neuron showing that PGE₂ (0.01-1 μM) depolarized the membrane in a dose-dependent manner. Note that in this cell, 0.01 μM of PGE₂ did not trigger spike firing. In this and the following figures, the downward deflections correspond to voltage responses to 300 ms hyperpolarizing pulses used to test the membrane input resistance. **D**, Dose-response curve of the PGE₂-induced membrane depolarization. The numbers of neurons tested at each dose is given in parentheses. Error bars indicate SEM. The EC₅₀ for the PGE₂-induced membrane depolarization was 0.018 μM, based on a logistic equation fitted to the data points. **E**, The excitatory effect of PGE₂ on GnRH-neurons persisted in the presence of the AMPA/kainate and NMDA receptors antagonists CNQX (20 μM) and DL-AP5 (100 μM) respectively. **F**, Loose patch-clamp recording showing the excitatory effect of PGE₂ on a GnRH neuron, characterized by a reversible acceleration of firing. Note that the effect was reproduced by a second application of PGE₂ to the same neuron. **G**, Loose patch-clamp recording showing the inhibitory effect of PGE₂ on a non-GnRH neuron located in the vicinity of GnRH-GFP neurons. The effect was characterized by a reversible slowing down of firing.

Figure 2. The PGE₂-induced activation of GnRH neurons is direct and involves an inward current. **A**, Whole-cell current-clamp recording showing that PGE₂ depolarized GnRH neurons in the presence of TTX (0.5 μM), CNQX (20 μM), DL-AP5 (100 μM), and bicuculline

(BIC, 20 μ M). Arrowheads indicate the time of application of hyperpolarizing and depolarizing current pulses to trace current-voltage relationships. **B1-B3**, Responses of a GnRH neuron to current injection from -70 pA to +70 pA before (**B1**, Control) and during application of PGE₂ (**B2**). The current-voltage relationship (**B3**) for the corresponding neuron obtained before (Control) and during the application of PGE₂ indicate that PGE₂ decreased the slope of the linear part of the curve, indicating a decrease in the membrane input resistance. Note that the two curves converged at -40 mV in this example. **C**, Whole-cell voltage-clamp recording showing that PGE₂ evoked an inward current in GnRH neurons in the presence of TTX (0.5 μ M), CNQX (20 μ M), DL-AP5 (100 μ M), and bicuculline (BIC, 20 μ M). The inward current was recorded at a holding potential of -70 mV. **D1-D2**, Current traces evoked by voltage ramps (duration: 12.5 s) from -120 mV to -30 mV at a holding potential of -70 mV in GnRH neurons. **D1**, Traces showing the current responses to the voltage ramp in GnRH neurons before (Control) and during application of PGE₂. **D2**, PGE₂-induced current obtained after subtracting the control from the PGE₂ curve. Note that the PGE₂-induced current is suppressed at -40 mV.

Figure 3. The PGE₂-induced activation of GnRH neurons is mediated by the EP2 receptor and requires the cAMP-PKA pathway. **A**, In a single GnRH neuron recorded with a whole-cell current clamp, the EP2 receptor agonist Butaprost evoked a membrane depolarization similar to that induced by PGE₂ whereas the EP1 receptor agonist 17-phenyl trinor PGE₂ (17PT-PGE₂) and the EP1-3 receptor agonist Sulprostone had no effect. Note that the discharge elicited by the Butaprost-induced membrane depolarization led to bursts of action potentials (asterisks). **B**, A bar graph illustrating the membrane depolarization in GnRH neurons induced by PGE₂, Butaprost, 17PT-PGE₂ and Sulprostone (*, $p < 0.05$ when compared with the membrane depolarization induced by PGE₂, one-way ANOVA; $n=4-10$ neurons). Error bars indicate SEM. **C1-C2**, Current traces evoked by voltage ramps (duration: 12.5 s) from -120 mV to -30 mV at a holding potential of -70 mV in GnRH neurons. **C1**, Traces showing the current response to the voltage ramp in GnRH neurons before

(Control) and during application of Butaprost, a selective EP2 receptor agonist. **C2**, Butaprost-induced current obtained after subtracting the control from the Butaprost curve. Note that the butaprost-induced current was suppressed at -35 mV in this example. **D**, EP2 receptor immunoreactivity (red) was detected in the cell body of GnRH-GFP neurons (green, arrow). Scale bar: 20 μm . **E**, Whole-cell current-clamp recording of a single GnRH neuron in the presence of TTX (0.5 μM) showing the effect of PGE₂ in the absence (top) and presence (bottom) of the membrane-permeable PKA inhibitor H-89. Note that H-89 attenuated the membrane depolarization and the decrease in membrane input resistance (downward deflections) induced by PGE₂. **F**, A bar graph illustrating the membrane depolarization induced by PGE₂ alone or in the presence of H-89 and two other membrane-permeable compounds, the more selective PKA inhibitor KT 5720 and the cAMP antagonist Rp-cAMPs (*, $p < 0.05$ when compared with the membrane depolarization induced by PGE₂ alone, one-way ANOVA; $n=3-11$ neurons). Error bars indicate SEM.

Figure 4. Astrocytic prostaglandin production sustains the electrical activity of GnRH neurons. Recordings of GnRH neurons were performed under whole-cell current-clamp using pipette solution 2 (ps 2, see Materials and Methods) to obtain a background of spontaneous activity from GnRH neurons. **A**, GnRH neurons showed spontaneous activity (Control), which was reduced after 10 min of perfusion with indomethacin (INDO, 50 μM), an inhibitor of the cyclooxygenases, enzymes responsible for prostaglandin production. **B**, INDO at a higher concentration (100 μM) strongly attenuated the spontaneous activity of GnRH neurons, accompanied by membrane hyperpolarization. Note that PGE₂ reversed the inhibitory effect of INDO. **C**, Bar graph illustrating the firing rate of GnRH neurons recorded in brain slices from wild-type mice (WT) exposed to INDO or fluoroacetate (FA), and from DN-erbB4 mice. (*, $p < 0.05$ when compared with the firing rate of GnRH neurons recorded in brain slices from WT mice, one-way ANOVA; $n=6-12$ neurons). Error bars indicate SEM. **D**, Pre-treatment of brain slices with fluoroacetate (FA, 5 mM, 60-120 min), a glial toxin, impaired the astrocytic uptake of sulforhodamine101 (SR101, red). Arrows show cells that took up SR101

in the vicinity of GnRH neurons (GFP, green) under control conditions (left panel). After FA treatment, very few cells were labeled with SR101 (right panel, arrowhead). Images were acquired at the level of the organum vasculosum of the lamina terminalis (OVLT). Scale bar: 100 μm . **E**, FA strongly reduced the firing rate of GnRH neurons. In this example, the slice was pre-treated with FA for 60 min and action potentials could be driven by a brief injection of a depolarizing current (asterisk). Note that PGE_2 retained its depolarizing effect. **F**, In DN-erbB4 mice, in which astrocytic PGE_2 release is diminished, most GnRH neurons were silent. In this recording, action potentials could be driven by a brief injection of depolarizing current (asterisk). Note that PGE_2 retained its depolarizing effect as well as reducing the membrane input resistance (downward deflections).

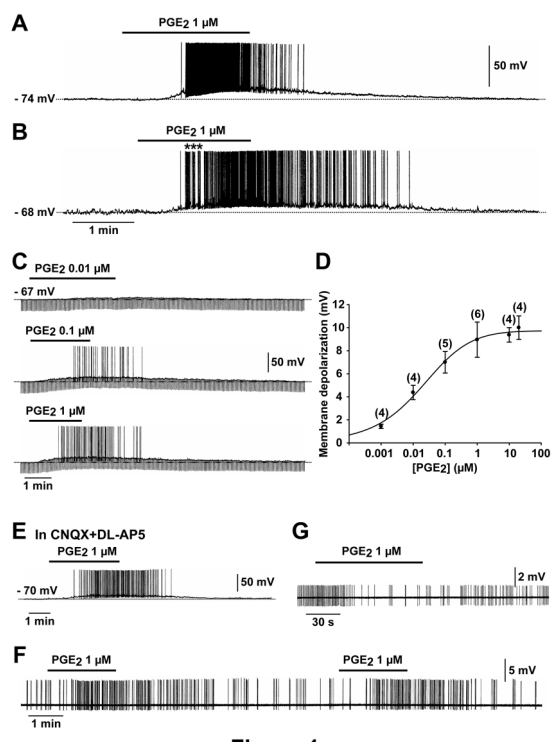


Figure 1

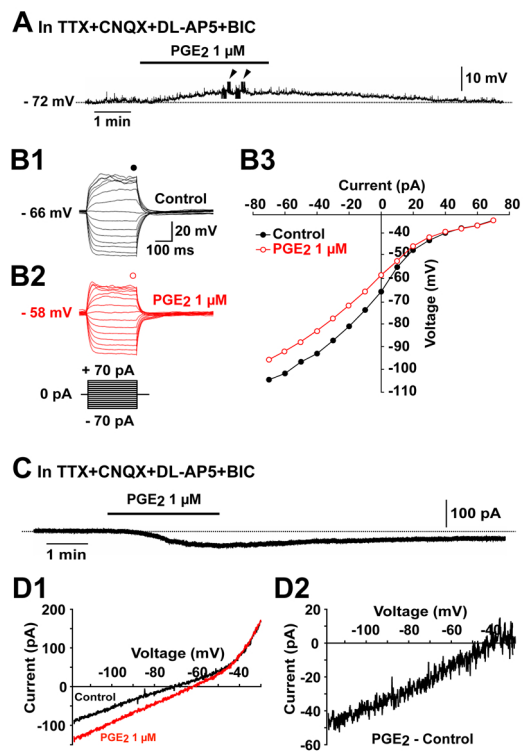


Figure 2

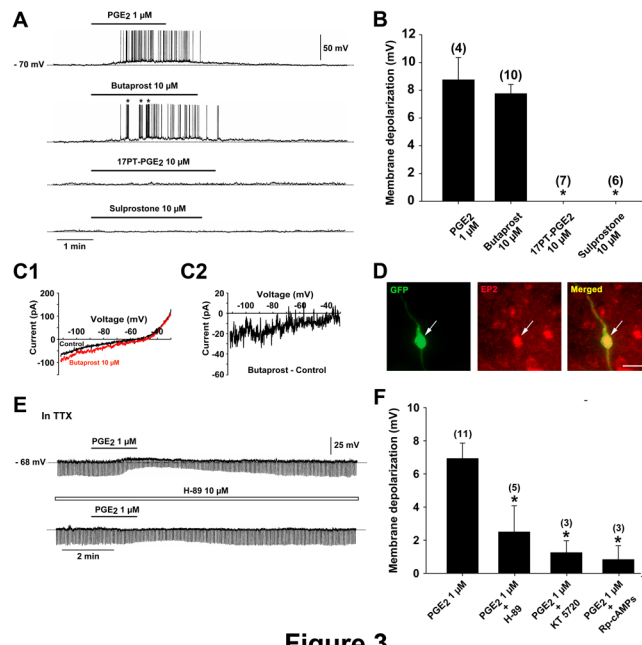


Figure 3

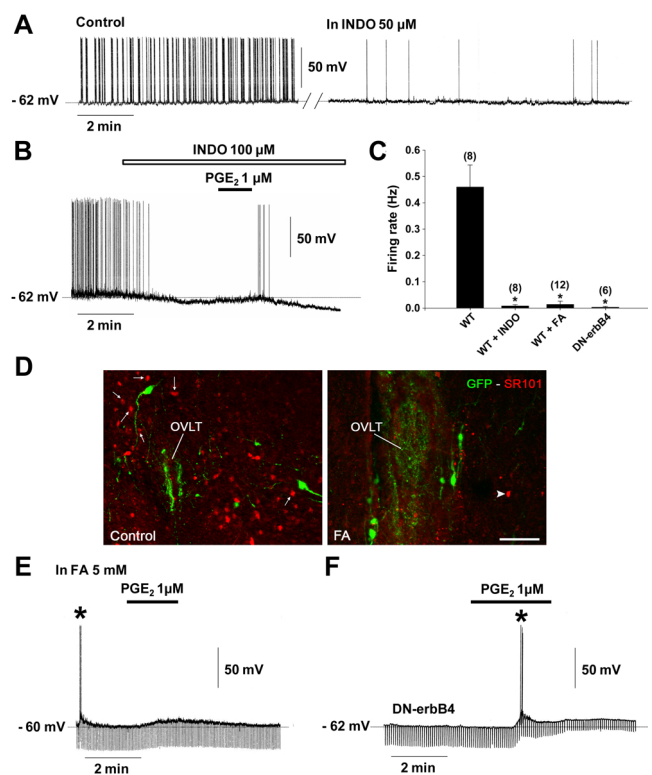


Figure 4