Prenatal androgens alter GABAergic drive to gonadotropin-releasing hormone neurons: Implications for a common fertility disorder

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Edited by Donald W. Pfaff, The Rockefeller University, New York, NY, and approved March 19, 2004 (received for review December 4, 2003)

Polycystic ovary syndrome, a fertility disorder affecting ≈7% of women, is characterized by elevated androgens, disrupted reproductive cycles, and high luteinizing hormone, the latter reflecting increased gonadotropin-releasing hormone (GnRH) release. In animal models, a similar reproductive endocrine phenotype occurs after prenatal androgen exposure. To study the effects of *in utero* androgen exposure directly on GnRH neurons, the central regulators of fertility, we prenatally androgenized (PNA) transgenic mice that express GFP in these cells. Pregnant females were injected with dihydrotestosterone, and their female offspring were studied as adults. PNA mice had irregular estrous cycles and elevated testosterone and luteinizing hormone levels, suggesting altered hypothalamo–pituitary–gonadal axis function. GnRH neurons receive a major input from γ-aminobutyric acid (GABA)ergic neurons, and GABA type A receptor activation may play a role in their regulation by steroids. We tested whether PNA alters GABAergic drive to GnRH neurons by comparing frequency and size of GABAergic postsynaptic currents in GnRH neurons from PNA and control females. Both postsynaptic current frequency and size were increased in PNA mice; these effects were reversed by *in vivo* treatment with the androgen receptor antagonist flutamide, suggesting that androgens mediated these effects. Changes in postsynaptic current frequency and size were action potential-independent, suggesting the possibility that PNA increased connectivity between GABAergic and GnRH neurons. The ability of prenatal steroid exposure to initiate changes that alter functional inputs to GnRH neurons in adults has important implications for understanding the regulation of normal reproduction as well as the hypothalamic abnormalities of fertility disorders.

Gonadotropin-releasing hormone (GnRH) neurons are the final common pathway for the central regulation of fertility. A pulsatile GnRH signal is required for secretion of the pituitary gonadotropins luteinizing hormone (LH) and follicle-stimulating hormone (FSH) (1), which drive steroidogenesis and follicular development during the female reproductive cycle. Steroids feed back to regulate GnRH pulse frequency. Variations in GnRH pulse frequency during the cycle are critical for the differential synthesis and release of LH and FSH; low frequency pulses favor FSH, and high frequencies favor LH (2, 3). During the luteal phase, progesterone negative feedback reduces GnRH pulse frequency (4–6), favoring FSH synthesis. FSH release is inhibited at that time by gonadal factors (7, 8), but low frequency GnRH release continues into the early follicular phase, when gonadal restraint on FSH release is removed. This produces a monotropic FSH rise in the early follicular phase that is critical for follicular maturation. In some forms of hypothalamic infertility, such as the common disorder polycystic ovary syndrome (PCOS), GnRH pulse frequency remains high, impairing this preferential release of FSH, and thus follicular maturation (9, 10). Evidence suggests that the high androgen levels characteristic of this disorder interfere with sensitivity to progesterone negative feedback (11); sensitivity is restored clinically by treatment with antiandrogens (12). Androgens may also have independent stimulatory effects on GnRH release.

In animal models, *in utero* androgen exposure disrupts fertility in adulthood in ways that resemble the reproductive endocrine phenotype of hyperandrogenemic human fertility disorders. This is postulated to be caused by androgen-induced reprogramming of neural development such that GnRH neurons are desensitized to steroid feedback (13, 14). Prenatally androgenized (PNA) adult female sheep and primates exhibit elevated LH pulse frequency (13) and irregular reproductive cycles (15, 16), evidence of disrupted regulation of the hypothalamic–pituitary–gonadal axis. To test the hypothesis that altered fertility in PNA animals occurs by means of a central mechanism at the GnRH neuron, we created a PNA model by using transgenic mice in which GnRH neurons are identifiable by expression of GFP (17), allowing effects of PNA to be studied directly on GnRH neurons.

Signals initiated by PNA may be communicated to GnRH neurons directly or transynaptically. A large body of work suggests that γ-aminobutyric acid (GABA)ergic afferents play a role in mediating steroid feedback (18–22). Although those studies could not examine the role of GABA directly on GnRH neurons, anatomical (23, 24) and functional (25–28) work indicates that direct regulation of GnRH neurons by means of the $GABA_A$ receptor ($GABA_AR$) occurs. GABA inhibits most mature neurons, but this is controversial for GnRH neurons. GnRH neurons maintain elevated chloride (29), as do other neurons derived from olfactory placode (30), and may thus be excited by $GABA_AR$ activation. Studies using functional approaches to examine GnRH neurons differ as to whether GABA excites or inhibits these neurons (refs. 29, 31, and 32 and S.M.M., S.D.S., and R. A. DeFazio, unpublished results), but direct correlations between reduced fertility and reduced GABAergic drive support the former (26, 27). Regardless of the response of GnRH neurons to $GABA_AR$ activation, $GABA_ARs$ clearly play a role in fertility regulation. We thus hypothesized that one mechanism by which prenatal androgens alter fertility is by altering GABAergic drive to these cells. Here, we compare reproductive cyclicity, hormone levels, and $GABA_AR$-mediated postsynaptic currents (PSCs), a measure of the endogenous synaptic GABAergic drive, in GnRH neurons from adult PNA and control females.

Materials and Methods

Animals. Adult transgenic female mice in which GFP is genetically targeted to GnRH neurons (17) were used. Mice were on a 14 h light/10 h dark cycle with lights on at 0500 EST, and maintained on standard rodent chow (Harlan Laboratories, Haslett, MI) and water ad libitum. Male and female mice were

This paper was submitted directly (Track II) to the PNAS office.

Abbreviations: GnRH, gonadotropin-releasing hormone; LH, luteinizing hormone; FSH, follicle-stimulating hormone; PSC, postsynaptic current; PNA, prenatally androgenized; GABA, γ-aminobutyric acid; TTX, tetrodotoxin; $GABA_AR$, $GABA_A$ receptor; PCOS, polycystic ovary syndrome.

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paired and females examined daily for copulatory plug (day 1 of pregnancy). On days 16–18 of pregnancy, mice were injected s.c. with 250 μg/day dihydrotestosterone (Sigma) or sesame oil vehicle; female offspring were studied as adults. This treatment period is a critical period for prenatal neural sexual differentiation in the rat, which has a similar developmental time course to the mouse (33, 34). In previous studies of PNA females, external genitalia were masculinized (13, 14, 35); a dose of dihydrotestosterone minimizing these virilizing effects was determined in pilot studies. Only PNA females with anal–genital distance similar to controls (5.6 ± 0.3 mm, control, $n = 10$; 6.3 ± 0.3 mm PNA, $n = 7$, $P > 0.1$) were used, suggesting that endocrine or reproductive changes in this model are not secondary to external phenotypic changes. Estrous cyclicity was assessed in all adult mice (4–6 months of age) by vaginal cytology. On the day of recording and blood sampling, all mice were in diestrus, and endocrine status was determined by measuring serum testosterone (Diagnostic Products, Los Angeles) and LH levels (36). A subset of adult mice was treated with the androgen receptor antagonist flutamide (Sigma, 10 mg/kg/day s.c. for 5–10 days before the day of experiment, or 10–15 days for monitoring estrous cyclicity). All procedures were approved by the Animal Care and Use Committee of the University of Virginia.

Slice Preparation and Recordings. All reagents were purchased from Sigma; 200-μm coronal sections through the preoptic area and hypothalamus were prepared as described (37, 38). For recording, individual slices were transferred to a recording chamber mounted on the stage of an Olympus BX50WI upright fluorescent microscope (Opelco, Dulles, VA). Slices in the recording chamber were continuously superfused with oxygenated recording saline kept at 30–32°C with an inline heating unit (Warner Instruments, Hamden, CT), with the addition of D(−)2-amino-5-phophonovaleric acid (APV, 20 μM) and 6-cyano-7-nitroquinoxaline-2,3-dione (10 μM) to block glutamatergic currents, and in some recordings tetrodotoxin (0.5 μM) to block action potentials. Experiments were performed by using PULSE CONTROL software (Instrutech, Mineola, NY), and currents were recorded with an EPC-8 amplifier (HEKA Electronics, Lambrecht, Germany), digitized by an ITC-18 acquisition interface (Instrutech) and stored by using IGOR PRO software (Wavemetrics, Lake Oswego, OR) on a G4 Macintosh computer.

Recording Postsynaptic Currents. Electrodes (2–4 MΩ) were filled with a high-chloride pipette solution with the addition of 4 mM MgATP and 0.4 mM NaGTP before adjusting to pH 7.2 with NaOH (26, 39). GFP–GnRH neurons were identified, and the whole-cell recording configuration was achieved. Membrane potential was clamped at −60 mV, and signals were filtered at 5–7 kHz with gain set at 10 mV/pA for 120-s recording periods. Liquid junction potential of 3 mV (40) was not corrected. PSCs were stored as Event Tracker files using PULSE CONTROL and IGOR PRO software. Input resistance (R_{in}), series resistance (R_s), and membrane capacitance (C_m) were continually monitored as described (41). Only recordings with $R_{in} > 500$ MΩ and $R_s < 20$ MΩ were included for analysis. Mean R_{in}, R_s, and C_m were not different ($P > 0.05$) among or within cells where comparisons were made. In a subset of recordings, elimination of all PSC activity after bath application of the $GABA_A$ receptor antagonist bicuculline (20 μM) confirmed that PSC events were $GABA_A$ receptor mediated. Individual cells were further examined to determine that changes in the PSC properties examined were not caused by alterations in passive properties or R_s within the acceptable ranges defined above.

PSC Analysis. Stored 120-s traces of current activity were analyzed offline by using custom event detection software to identify PSCs (events). Threshold for event detection was set manually for each 120-s record and was typically ≥5 pA. Events were confirmed by eye, and detection errors were corrected manually. Mean event frequency (in Hz) from at least three 120-s records was calculated for each cell to obtain mean PSC frequency in each treatment group. Group means were compared by using one-way ANOVA, followed by post hoc analysis with Fisher's protected least significant difference test and Student–Newman–Keuls test for pairwise comparisons when appropriate ($P < 0.05$). Averaged PSC waveforms were generated for each cell after aligning events on the rising phase and were used to illustrate differences in PSC amplitude. Averaged PSCs were then normalized by amplitude to show differences in decay time. Rate of rise, peak amplitude, and 10–90% decay time were determined for every event. To compare PSCs among treatment groups, probability distributions for each parameter in each treatment group were generated by using 100 randomly selected events per cell or all events if <100 occurred. To compare PSCs recorded from a single cell before and after *in vitro* treatment with tetrodotoxin (TTX), probability distributions for each parameter were generated during baseline and treatment periods using all events recorded during the respective period, as described (26, 42). Probability distributions were compared between and within cells with the Kolmogorov–Smirnov Goodness of Fit test (SPLUS PROFESSIONAL 2 data analysis software, MathSoft, Cambridge, MA). Mean values for each PSC parameter for each cell were then used to calculate mean percent change from controls. Means for each PSC parameter were compared among treatment groups using one-way ANOVA followed by post hoc analysis with Fisher's protected least significant difference and Student–Newman–Keuls for pairwise comparisons when appropriate. All values are reported as mean ± SEM, and significance was set at $P < 0.05$.

Results

PNA Disrupts Estrous Cyclicity in Adulthood by Means of Androgen Receptor D-Dependent Mechanism(s). In PNA females, estrous cycle length (20.9 ± 3.0 days) was lengthened compared to controls (5.9 ± 0.3 days, $P < 0.001$, Fig. 1). This was largely due to increased duration of estrus in PNA mice ($P < 0.001$). Interestingly, treatment with the androgen receptor antagonist flutamide (10 mg/kg/day) restored normal estrous cyclicity to PNA females such that cycle length (5.1 ± 0.4 days, $n = 6$, $P > 0.5$) and the percentage of time spent in estrus were no longer different from controls ($P > 0.3$). Flutamide had no effect on the cycle length in control mice (5.6 ± 0.6 days, $n = 4$, $P > 0.04$, not shown).

Testosterone and LH Levels in PNA Females. To examine the endocrine milieu contributing to abnormal cycles in PNA mice, we measured LH and testosterone. In adult PNA female mice on diestrus, serum LH and testosterone levels were elevated compared to the levels seen in control diestrus females ($n = 6$ each, $P < 0.05$, Fig. 2). These data suggest PNA reprogrammed the output of the hypothalamic–pituitary–gonadal axis in adults.

GABAergic PSC Frequency Is Increased in PNA Females by Means of Androgen Receptor-Dependent Mechanism(s). To begin to investigate the central mechanisms underlying such reprogramming, we tested whether PNA alters GABAergic drive to GnRH neurons. The frequency of PSCs, a measure of presynaptic drive, was markedly increased in PNA compared to control females (control, $n = 6$ cells, PNA, $n = 17$, $P < 0.05$, Fig. 3). To determine whether the elevated androgens in PNA mice contributed to this, PNA and control mice were treated *in vivo* with the androgen receptor antagonist flutamide. Flutamide did not alter PSC frequency in control females (0.4 ± 0.2 Hz, control $n = 6$, 0.2 ± 0.1 Hz, control plus flutamide, $n = 4$, $P > 0.3$). In PNA females,

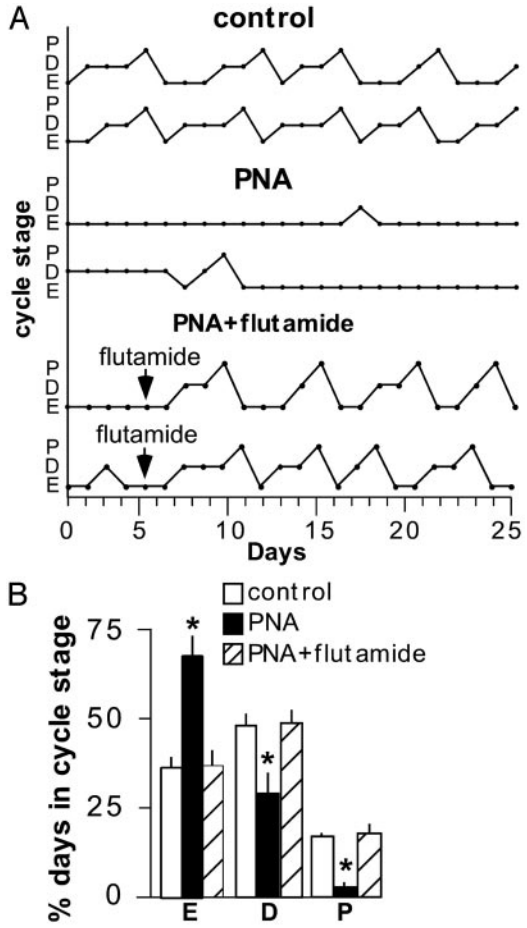

Fig. 1. PNA disrupts estrous cyclicity in adulthood. (*A*) Representative estrous cycles in control (*Upper*), PNA, and PNA plus flutamide mice (*Lower*). Arrow indicates time flutamide treatment was begun. (*B*) Percent of days spent in each estrous cycle stage (E, estrus; D, diestrus; P, proestrus) in PNA, control, and PNA plus flutamide mice. *, $P < 0.05$ versus control.

however, flutamide restored PSC frequency to control levels ($n = 8$, $P < 0.03$ vs. PNA, $P > 0.7$ vs. control, Fig. 3), suggesting that the increase in GABAergic drive to GnRH neurons in these

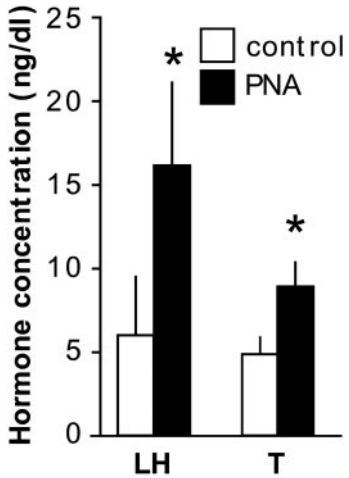

Fig. 2. PNA elevates LH and testosterone (T) levels in adult diestrus mice ($n = 6$ control; $n = 6$ PNA). *, $P < 0.05$ versus control.

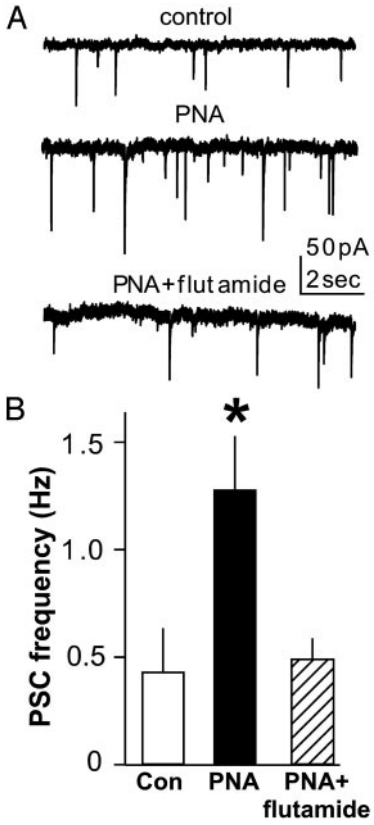

Fig. 3. PNA increased afferent GABAergic drive to GnRH neurons. (*A*) Recordings of GABAergic PSCs from a representative GnRH neuron from a control female (*Top*), a PNA female (*Middle*), and a PNA female treated *in vivo* with flutamide (*Bottom*). (*B*) Mean ± SEM PSC frequency in GnRH neurons from control ($n = 6$ cells), PNA ($n = 17$), and PNA plus flutamide ($n = 8$) adult females. *, $P < 0.05$ versus control (Con).

animals occurred by means of androgen receptor-dependent mechanism(s).

PSC Size Is Increased in PNA Females by Means of Androgen Receptor-Dependent Mechanism(s). In addition to PSC frequency, we also examined the size of PSCs to determine whether PNA alters the responsiveness of GnRH neurons to $GABA_AR$ activation. We compared three parameters of PSC size: rate of rise (a measure of receptor on-rate), amplitude (a measure of conductance), and decay time (a measure of receptor off-rate/affinity). All parameters were increased in PNA compared to control mice ($P < 0.04$ vs. control, Fig. 4). Similarly to the effect of flutamide on PSC frequency, *in vivo* flutamide treatment to control females did not alter any of these PSC parameters ($P > 0.1$ vs. control for rate of rise, amplitude, and decay time, not shown). In contrast, flutamide treatment of PNA animals restored PSC rate of rise, amplitude, and decay time back to control levels, indicating that androgens were responsible for altered PSC size in these females ($P > 0.6$ vs. control, $P < 0.03$ vs. PNA for each parameter, Fig. 4).

The Effects of PNA on GABAergic Drive Are Activity-Independent. To begin to study possible mechanisms for increased GABAergic PSC frequency and size in PNA mice, we compared PSCs before and after *in vitro* treatment with tetrodotoxin (0.5 μM), applied directly to the brain slice by means of the external recording solution. TTX eliminates action potential-dependent effects, thus eliminating changes caused by altered firing activity of the presynaptic cell. Within individual GnRH neurons from PNA

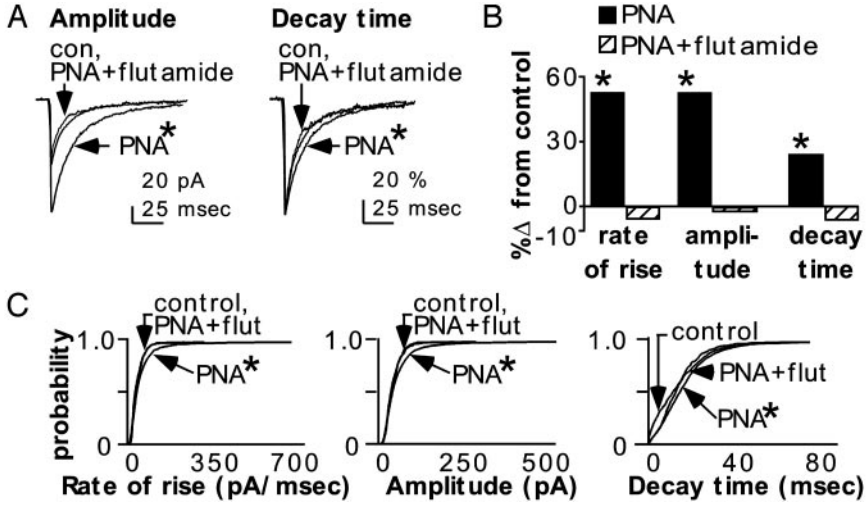

Fig. 4. PNA increased the efficacy of $GABA_A$ receptor activation in GnRH neurons. (*A*) Averaged (*Left*) and normalized averaged (*Right*) PSC traces from representative GnRH neurons from control, PNA, and PNA + flutamide females show differences in PSC amplitude and decay time, respectively, among the groups. (*B*) Percent change of mean values of PSC rate of rise, amplitude, and decay time in GnRH neurons in PNA and PNA + flutamide females from control values. (*C*) Probability distributions of PSC rate of rise (*Left*), amplitude (*Center*), and decay time (*Right*), created by using all PSC events from all cells in each treatment group, show among-group comparisons for these PSC properties. *, $P < 0.05$ versus control.

mice, *in vitro* treatment with TTX had no effect on PSC frequency (1.2 ± 0.3 Hz, pre-TTX, 1.2 ± 0.3 Hz, +TTX, $P > 0.4$, $n = 8$). Similarly, PSC size was not altered by TTX (rate of rise, $P > 0.4$; amplitude, $P > 0.3$; decay time, $P > 0.1$, Fig. 5). Together, these data suggest that increased GABAergic drive to GnRH neurons in PNA mice occurred by means of activity-independent mechanism(s).

Discussion

The feedback regulation of GnRH neurons by steroid hormones is critical to normal reproductive function and occurs in part through altered GABAergic drive to these cells (18–22, 28, 37). Altered sensitivity to steroid feedback is associated with forms of hypothalamic infertility such as the common reproductive disorder PCOS, a leading cause of infertility in women. In the present study, we show that prenatal androgenization, a treatment that can recapitulate the reproductive neuroendocrine phenotype of such disorders, induces organizational and/or activational changes during development to increase frequency of GABAergic drive to GnRH neurons in adults, as well as the responsiveness of GnRH neurons to $GABA_AR$ activation. This finding suggests that the GABAergic system may play a role in forms of hypothalamic infertility.

Ideally, animal models of human disease model the majority of clinical characteristics of that disorder. In this respect, hyperandrogenemia associated with elevated activity of the hypothalamic–pituitary axis in women is associated with elevated LH levels and disrupted cyclicity, which were exhibited both by the PNA mice in the present study and by previous PNA models in other species (13, 14). Similarly to clinical phenotypes, PNA mice in this study also exhibited elevated androgen levels; androgens were either normal (13) or unreported (14) in previous animal studies. Thus, in terms of reproductive endocrine phenotype, PNA mice used in this study parallel the human condition. It is important, however, to recognize the limitations of such a model. For example, the small blood volume of mice makes careful characterization of hormone secretory patterns extremely difficult; our data are thus based on single terminal samples. Furthermore, mice are a polyovulatory species, and future comparison of potential ovarian phenotypes, such as cyst formation, must be done with caution. Finally, although the endocrine changes of the murine estrous cycle parallel those of the human ovulatory cycle, there are substantial differences in length of the various phases, as well as in hormone levels. Despite these caveats, the similarities between the reproductive endocrine phenotypes of PNA mice and women with hyperandrogenemia-associated infertility suggest cellular studies of underlying neural mechanisms in animal models may shed light on possible etiologies of human disease. Of further interest with regard to the usefulness of PNA animal models is a recent observation that, during pregnancy, women with PCOS maintain elevated androgens, thus potentially exposing their offspring (43).

Development of the PNA model in GnRH–GFP mice allows electrophysiological analyses of the central mechanisms for these reproductive changes to be performed directly at the GnRH neuron. Possible alterations in GABAergic drive were investigated because anatomical (23, 24) and functional (25–28) evidence indicates GABAergic neurons play a role in the synaptic regulation of GnRH neurons. Both frequency and size of GABAergic PSCs were increased in PNA females, suggesting that one mechanism for reproductive dysfunction in these animals may be altered GABAergic drive to GnRH neurons. Interestingly, LH was also elevated in PNA mice, providing further physiological support for electrophysiological evidence suggesting an excitatory action of GABA directly at the GnRH neuron is possible (26, 27, 29).

Increased PSC frequency in PNA mice may result from a number of mechanisms, including increased activity of GABAergic neurons afferent to GnRH neurons, increased efficiency of vesicle docking/release in afferent neurons, and/or increased connectivity between these cell types. In PNA animals, PSC frequency was not altered by inhibition of action potential firing by tetrodotoxin, suggesting the frequency increase occurred by means of an activity-independent mechanism. Frequency of PSCs recorded in tetrodotoxin has been shown to be proportional to the synaptic contacts (44). The failure of tetrodotoxin to alter PSC frequency is thus consistent with the hypothesis that PNA increased connectivity between GABAergic and GnRH neurons. Although this hypothesis awaits further testing, steroid hormones alter synaptic connections in adults (45, 46). In GnRH neurons specifically, removal of endogenous androgens by castration reduced spine number on these cells in males (47). Additionally, in female

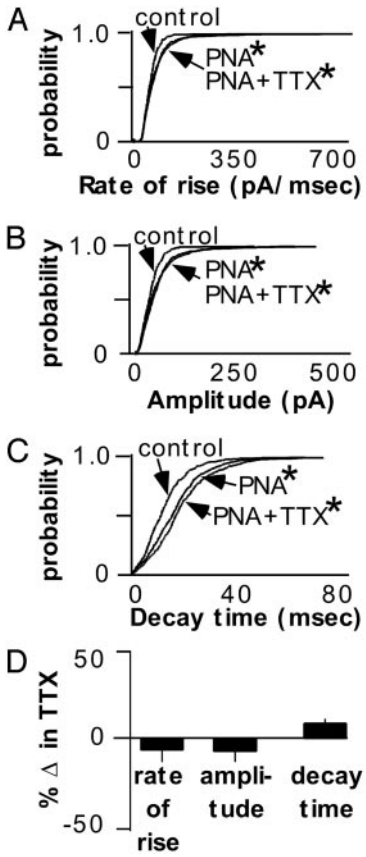

Fig. 5. Effects of PNA on PSC size are action potential-independent. (*A–C*) Probability distributions of PSC rate of rise (*A*), amplitude (*B*), and decay time (*C*) show comparisons for these PSC properties in GnRH neurons from PNA females before and after *in vitro* treatment with TTX (0.5 μM, PNA + TTX). Distributions for each PSC parameter are also shown for GnRH neurons from control mice for comparison. (*D*) Percent change from baseline after *in vitro* treatment with TTX in PSC rate of rise, amplitude, and decay time in GnRH neurons from PNA females. *, $P < 0.05$ versus control (*A–C*) or from baseline (*D*).

sheep, prenatal androgenization reduced synaptic contacts with GnRH neurons to levels seen in males (48). Although the direction of change in that study was in the opposite direction to what might be predicted from the present work, neurochemical phenotype of those synapses was not determined; thus, it remains possible that a relative increase in GABAergic connections occurred. Together with the present data, these studies indicate that androgens may help determine the connectivity between GnRH and afferent neurons and suggests that this is a possible mechanism mediating increased PSC frequency in PNA females.

Increased PSC size indicates PNA also increased the efficacy of $GABA_AR$ activation in GnRH neurons. As with the effect of androgenization on PSC frequency, effects on PSC size were not altered by blocking action potentials, again suggesting activity-independent mechanisms were responsible for this androgen action. Possibilities include increased vesicle size or concentration in the presynaptic neuron and/or changes to the postsynaptic GnRH neuron to increase the response to GABA. The latter could be due to androgen receptor activation in GnRH neurons, but these receptors have only rarely been detected in GnRH neurons (49, 50), making direct action through this mechanism less likely. Because the observed changes took place in a whole-animal model, however, it is important to bear in mind that androgens could have acted presynaptically to induce changes in other neurotransmitter or neuromodulator systems that could impact gene expression in GnRH neurons. In this regard, androgens alter $GABA_AR$ subunit expression (51, 52); because the particular combination of subunits determines the efficacy of activation, such a mechanism might account for the changes observed. Another possibility is altered expression of kinases or phosphatases within GnRH neurons that alter $GABA_AR$ function (53, 54). Androgens could also be converted to neural steroid derivatives. The androgen derivative dehydroepiandrosterone sulfate (DHEAS) is an allosteric $GABA_AR$ antagonist (55) and, contrary to the androgen action reported here, decreases the response of GnRH neurons to $GABA_AR$ activation (39), suggesting that any action of neural androgen derivatives is not dominant in determining PSC size. Regardless of the particular mechanism(s) involved, increased PSC size means a larger chloride current flow through activated $GABA_ARs$, which could induce larger depolarizations in membrane potential. This would increase the probability that membrane potential will pass the threshold for action potential firing, leading to increased firing activity and, ultimately, hormone release in PNA mice. The elevated LH levels in these mice are consistent with this interpretation.

Effects on both frequency and size of PSCs were reversed when PNA mice were treated *in vivo* with the androgen receptor antagonist flutamide. Importantly, reversal of these effects by flutamide, in addition to the fact that prenatal androgenization was induced by treating with dihydrotestosterone, which cannot be converted to estrogens, provides strong evidence that effects of prenatal androgenization on GABAergic drive to GnRH neurons were androgen receptor mediated. Interestingly, flutamide treatment also restored normal estrous cyclicity to PNA females, suggesting a normalization of hypothalamo-pituitary axis activity, in addition to restoration of normal GABAergic drive at the level of the GnRH neuron. This finding is consistent with the hypothesis that androgen-induced alterations in GABAergic signaling are at least one cause of irregular reproductive cyclicity in this model. Further, the observation that treating adult PNA animals can restore abnormalities in the reproductive neuroendocrine axis initiated prenatally suggests that if prenatal androgen exposure is an etiology for PCOS, this disorder should be medically treatable in adult women.

In women with PCOS, sensitivity to steroid feedback is restored by flutamide treatment, which suggests that elevated androgens contribute to GnRH neuron hyperactivity in this disorder (12). Of interest, it was recently shown that central GABA levels are elevated in PCOS patients[§] and that upregulation of GABA expression near GnRH neurons induces a PCOS-like phenotype in rats (56). Together, these data suggest a working hypothesis that increased GnRH pulse frequency in PCOS may be attributable to androgen-induced increases in GABAergic drive to GnRH neurons. Determining the role GABA plays in the reproductive abnormalities seen in PCOS, as well as other disorders that affect fertility, including catamenial epilepsy in which fertility is decreased in the face of aberrant central GABAergic signaling (57), will be important for determining the etiologies of and improving prevention and treatment strategies for these conditions, as well as to understanding the central control of normal reproduction.

[§] Loucks, T. L., Rohan, L. C., Kalro, B. N. & Berga, S. L. (2002) in *Endocrine Society 84th Annual Meeting Programs & Abstracts* (Endocrine Society Press, Bethesda), pp. 108–109 (abstr.).

We thank Dr. Xu-Zhi Xu for technical assistance and Dr. John C. Marshall for editorial comments. This work was supported by National Institutes of Health/National Institute of Child Health and Human Development Cooperative Agreement U54 HD28934 and the U54 Ligand and Assay Core. S.D.S. was supported by National Institutes of Health/National Institute of Child Health and Human Development Cooperative Agreement F31 NS43871.

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