

## Comments

# Do $\alpha$ -Adrenergic Mechanisms Regulate Spontaneous or Opiate-Modulated Pulsatile Luteinizing Hormone Secretion in Man?\*

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**ABSTRACT.** Brain noradrenergic mechanisms participate in the excitatory control of episodic LH release in many experimental animals, including the nonhuman primate. In addition, augmentation of pulsatile LH release in the rodent in response to opiate receptor antagonists is dependent upon intact central noradrenergic pathways. The applicability of these tenets to humans is not known. We tested the excitatory influence of brain noradrenergic systems on pulsatile LH secretion in normal men by administering phenoxybenzamine (an irreversible, preferentially postsynaptic,  $\alpha_1$ -receptor blocker) or  $\alpha$ -methyldopa (an inhibitor of brain adrenergic transmission). Five normal men underwent repetitive (every 20 min) venous sampling for 8 h to characterize episodic LH release quantitatively under basal conditions and after the administration of naltrexone, a potent

opiate receptor antagonist which stimulates pulsatile LH release. Subjects received saline, phenoxybenzamine (1 mg/kg, iv, over 90 min), or  $\alpha$ -methyldopa (250 mg, orally, every 6 h). The following parameters of spontaneous episodic LH secretion were not altered after phenoxybenzamine or  $\alpha$ -methyldopa administration: mean and integrated serum LH concentrations, LH pulse frequency, LH pulse amplitude (percentage or milliinternational units per ml increment), and absolute peak serum LH values. In addition, the administration of adrenergic inhibitors did not impede the capacity of naltrexone to significantly augment pulsatile LH secretion in these subjects. We conclude that in the doses used, phenoxybenzamine and  $\alpha$ -methyldopa do not alter spontaneous or opiate-modulated episodic LH release in normal men. (*J Clin Endocrinol Metab* 57: 1292, 1983)

**T**HE ROLE of excitatory noradrenergic mechanisms in stimulating pulsatile secretion of LH in man is not known. Thus, we posed the following two questions. 1) Is basal (spontaneous) pulsatile LH secretion in normal men altered by the administration of noradrenergic antagonists? For this purpose, we used two pharmacological agents (phenoxybenzamine and  $\alpha$ -methyldopa) that can attenuate LH release in menopausal women or nonhuman primates (1-3). 2) Is the increase in pulsatile LH secretion induced by opiate receptor antagonism in normal men mediated by noradrenergic mechanisms? To address this issue, we employed a potent opiate receptor antagonist, naltrexone, which reliably stimulates pulsatile LH secretion in normal men (4).

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## Materials and Methods

### Subjects

Five men (aged 21-25 yr) of normal body weight participated. The study was approved by the Human Investigations Committee of The University of Virginia, and written informed consent was obtained. Each man had a normal physical examination (including testicular size) and a normal sexual developmental history. Basal serum T<sub>4</sub>, TSH, PRL, LH, FSH, testosterone, and estradiol concentrations were within normal limits.

### Test procedure

Subjects were admitted to the Clinical Research Center on three occasions, each of 2 days duration. Admissions were separated from one another by at least 6 weeks. On each admission, an indwelling heparin lock needle was inserted into a forearm vein, and blood samples were withdrawn every 20 min for 8 h, beginning at 0900 h (at least 1 h after placement). Due to the very long half-life of naltrexone [ $\sim$ 10 h (5)], the placebo elixir was given on the first day and naltrexone (1.0 mg/kg; as a 10 mg/ml elixir, orally; Endo Laboratories, Montreal, Canada) was given on the second day. On any given admission, subjects received placebo,  $\alpha$ -methyldopa, or phenoxy-

ybenzamine (see below). Each subject served as his own control.

When phenoxybenzamine was given, it was infused at a dose of 1.0 mg/kg, iv, over 90 min beginning at 0900 h. Before drug infusion, we obtained 2 h of additional basal venous sampling from 0700–0900 h. Placebo or naltrexone was given 30 min after the start of drug infusion.  $\alpha$ -Methyldopa (250 mg, orally) was given every 6 h, beginning 1 day before venous sampling, and this dose was continued during placebo or naltrexone administration.

#### *Sample processing and immunoassays*

Blood samples were withdrawn through the indwelling needle after first removing the heparin solution with a separate syringe. All samples were allowed to clot at room temperature before centrifugation. Serum was removed and stored at  $-20^{\circ}\text{C}$  until assay. All samples from an individual were analyzed in the same assay to avoid interassay variability.

Serum PRL,  $T_4$ , TSH, testosterone, and estradiol were determined as previously described (6). Serum LH concentrations were measured in triplicate using a modification of the method of Odell *et al.* (7), using reagents described previously (6). Seven additional pools of serum were assayed nine times each to define the intraassay variability more precisely at multiple points along the displacement curve. The analysis of pulsatile secretion employed intraassay variance specific to the relevant level of displacement (see below). The intraassays coefficients of variation were between 6.5–8.5% for the range of measured LH concentrations in the present study.

#### *Quantitative analysis of pulsatile hormone secretion*

Quantitative analysis of pulsatile hormone secretion employed the computerized methods of Santen and Bardin (8) and Clifton and Steiner (9). We used the classical algorithm of Santen and Bardin to calculate individual pulse amplitudes (expressed as the percent increase above the preceding nadir) and area under the 8-h time-concentration curve (8). To identify individually significant hormone fluctuations (pulses) that exceeded the variance associated with procedural and assay noise, we applied the program of Clifton and Steiner (9). This method identifies individual peaks, computes mean pulse frequency (and periodicity), and calculates mean incremental pulse amplitude above the preceding nadir. LH pulses are detected by sequential scanning of data using an adaptive threshold, which is initially estimated as 2.7 times the within-assay SD (9). This procedure is effective at signal to noise ratios of 1.5 or greater. In the present study, LH pulses exhibited a signal to noise ratio of at least 2.5. Individual pulses detected by the method of Clifton and Steiner (9) were 83% concordant with those defined by the method of Santen and Bardin (8) (Veldhuis, J. D., unpublished data). When a single prominent LH pulse was apparent (amplitude exceeding that of other LH pulses by  $>50\%$ ), the data were rescanned after omission of the dominant pulse to obviate damping of the residual pulse signals. The program was modified to display the individual pulses identified.

#### *Statistical analyses*

Data are presented as the mean  $\pm$  SD. Student's paired two-tailed *t* testing was used to assess within-subject effects. Exact *P* values were computed with the aid of the general statistics library module of a Texas Instruments (TI-59, Houston, TX) desk calculator.

## Results

#### *Control conditions*

In the absence of noradrenergic inhibitors, the administration of naltrexone significantly increased mean serum LH concentrations, area under the LH concentration *vs.* time curve, LH pulse frequency, and peak LH pulse amplitude (Table 1, upper panel Control). These data confirm our earlier description of the stimulatory effect of naltrexone on pulsatile LH release (4).

#### *$\alpha$ -Methyldopa administration*

The parameters of pulsatile LH secretion after the ingestion of  $\alpha$ -methyldopa were similar to those obtained under control conditions in each man. In addition, when naltrexone was administered to subjects receiving  $\alpha$ -methyldopa, a significant increase in pulsatile gonadotropin secretion still resulted. This increase was reflected in significantly higher mean and integrated serum LH concentrations, increased LH pulse frequency, and augmented absolute peak LH amplitude (Table 1). All of the five subjects had a stimulatory response to naltrexone after  $\alpha$ -methyldopa administration. Representative profiles of pulsatile LH secretion are shown for one subject in Fig. 1.

#### *Phenoxybenzamine administration*

After the iv infusion of phenoxybenzamine, standing blood pressure fell to less than 80 mm Hg for 8–24 h in each subject, reflecting profound peripheral  $\alpha$ -adrenergic blockade. However, supine blood pressure was not altered in any subject. Under these conditions of effective  $\alpha$ -receptor antagonism, quantitative characteristics of LH secretion were not affected in any subject (Table 1). Moreover, the administration of naltrexone still significantly ( $P < 0.01$ ) augmented parameters of pulsatile LH secretion in these individuals. A typical profile of pulsatile LH release during and after phenoxybenzamine infusion is shown in Fig. 2.

## Discussion

Extensive studies in experimental animals such as the rodent, domestic hen, ewe, and nonhuman primate (1, 2) have disclosed significant excitatory influences of postsynaptic noradrenergic pathways on spontaneous pulsa-

TABLE 1. Influence of phenoxybenzamine or  $\alpha$ -methyldopa administration on pulsatile gonadotropin secretion in normal men

Treatment	Mean LH <sup>a</sup>	Area <sup>b</sup>	Pulses/8 h	Pulse amplitude characteristics			Mean periodicity <sup>f</sup>
				Incremental <sup>c</sup>	Peak <sup>d</sup>	Fractional <sup>e</sup>	
I. Control							
Placebo (P)	4.89 ± 1.6	2347 ± 602	3.3 ± 0.67	3.7 ± 0.9	6.1 ± 1.7	90 ± 24	135 ± 37
Naltrexone (N)	6.24 ± 1.0	2982 ± 641	4.6 ± 0.75	3.0 ± 1.3	8.0 ± 1.8	101 ± 33	96 ± 18
P value (P vs. N)	0.003	0.02	0.005	NS	0.004	NS	0.005
II. $\alpha$ -Methyldopa + P							
$\alpha$ -Methyldopa + P	4.06 ± 1.20	1950 ± 593	3.4 ± 0.80	2.22 ± 0.82	5.32 ± 1.57	73 ± 18	137 ± 26
$\alpha$ -Methyldopa + N	5.84 ± 1.33	2735 ± 686	4.8 ± 0.75	3.66 ± 1.80	7.52 ± 2.03	83 ± 37	86.6 ± 14
P value (P + $\alpha$ -methyldopa vs. N + $\alpha$ -methyldopa)	0.007	0.02	0.002	NS	0.006	NS	0.001
III. Phenoxybenzamine + P							
Phenoxybenzamine + P	5.52 ± 1.03	2644 ± 494	3.6 ± 0.8	5.22 ± 2.31	7.44 ± 1.43	104 ± 35	127 ± 29
Phenoxybenzamine + N	6.73 ± 0.81	3231 ± 397	5.0 ± 1.1	5.50 ± 2.53	9.62 ± 1.92	91 ± 38	88 ± 11
P value (P + phenoxybenzamine vs. N + phenoxybenzamine)	0.003	0.004	0.002	NS	0.002	NS	0.008

All data are the mean ± SD derived from five normal men. P, Placebo; N, naltrexone.

<sup>a</sup> Milliinternational units per ml (mean ± SD; n = 25 samples).

<sup>b</sup> Area in milliinternational units per ml × min (over 8 h of sampling).

<sup>c</sup> mIU/ml increment from nadir to peak.

<sup>d</sup> Maximal absolute LH value achieved in the pulse (milliinternational units per ml).

<sup>e</sup> Percent increase above nadir.

<sup>f</sup> Minutes.

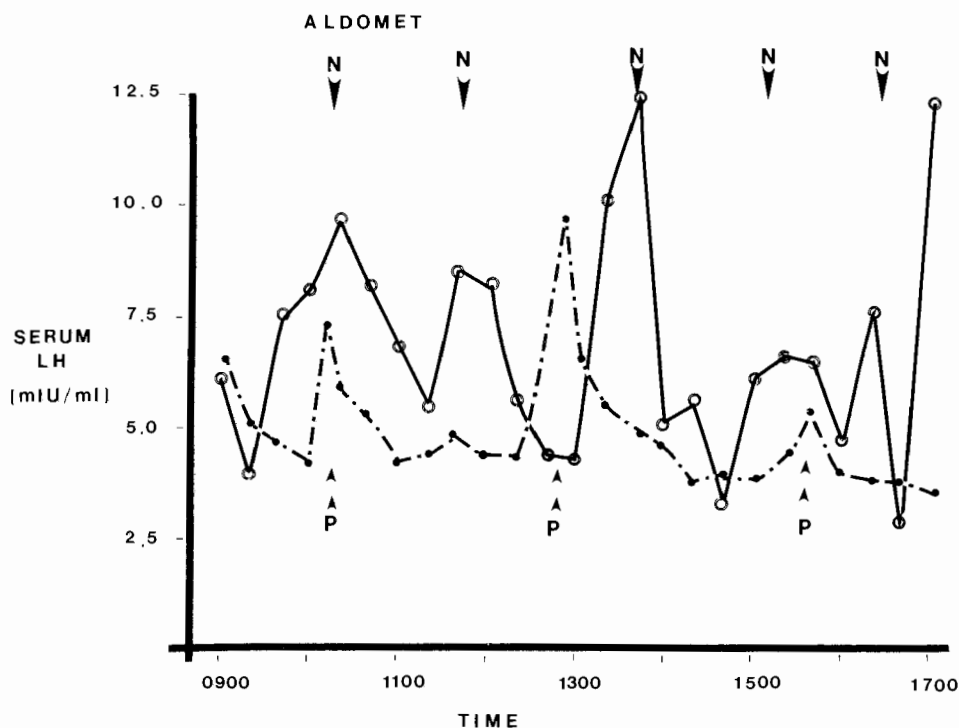


FIG. 1.  $\alpha$ -Methyldopa (ALDOMET) did not suppress pulsatile LH secretion in normal men. Results are illustrated for one of the five men. P, Pulses of LH during placebo ingestion (— — —); N, pulses of LH during naltrexone (—). Serum LH concentrations were measured in samples drawn at 20-min intervals from 0900–1700 h.

tile LH release (reviewed in Ref. 10). For example, in the rhesus monkey, treatment with the  $\alpha$ -receptor antagonists phentolamine and phenoxybenzamine abolished episodic LH release (1, 2). Moreover, there is evidence that brain adrenergic mechanisms in the rodent also mediate

the capacity of opiate receptor antagonists to augment pulsatile LH secretion (11, 12). In contrast, investigations in man are limited by the following considerations: 1) effects of the potent, irreversible, and preferentially postsynaptic  $\alpha$ -receptor antagonist phenoxybenzamine

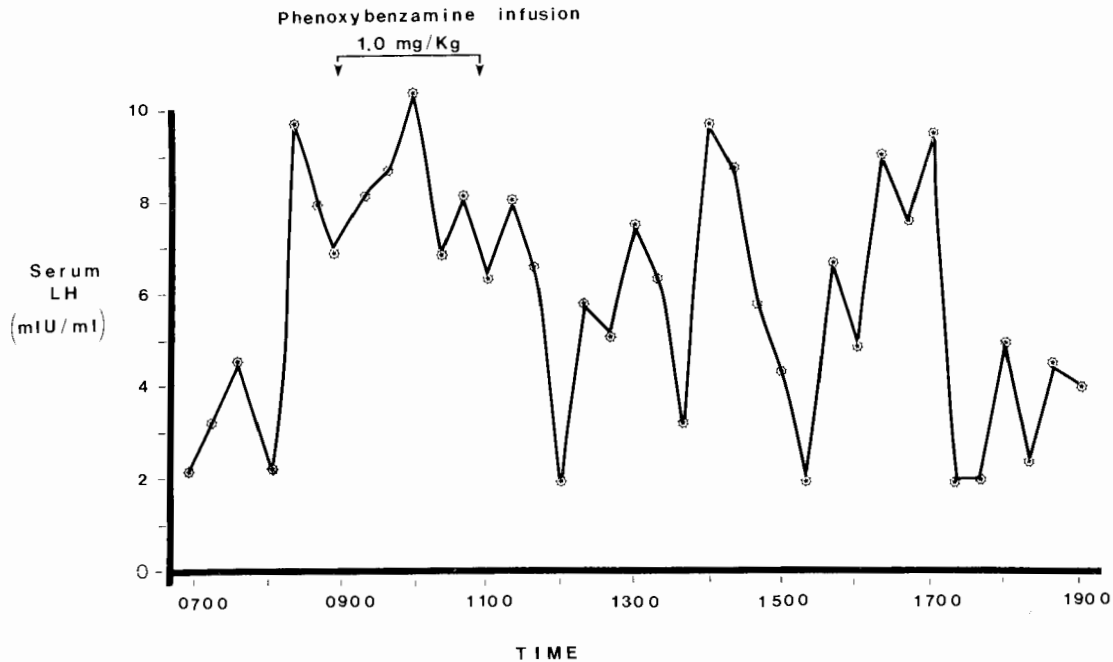


FIG. 2. Phenoxybenzamine did not inhibit spontaneous (basal) pulses of LH in normal men (data enumerated in Table 1). Prominent fluctuations in LH levels occurred during infusion of the drug, as illustrated by these results for one of the five men. Blood was drawn at 20-min intervals from 0700–1900 h, and phenoxybenzamine was infused from 0900–1030 h.

on LH secretion have not been studied in man; and 2) whether noradrenergic mechanisms participate in opioid-modulated LH secretion in the human is not known.

Based upon these considerations, we employed a dose of phenoxybenzamine (1 mg/kg, iv) which 1) equals or approximates that which effectively blocked pulsatile LH release in the monkey (1 and 3 mg/kg, iv), and 2) achieved unequivocal blockade of at least peripheral  $\alpha$ -receptors in these normal men. We do not know whether central (brain) noradrenergic blockade was attained to the same degree (see below). However, under these conditions, quantitative properties of basal pulsatile gonadotropin secretion remained indistinguishable from control values in the same subjects. In particular, mean and integrated serum concentrations of LH, LH pulse frequency, and LH pulse amplitude characteristics were all independent of peripheral inhibition of  $\alpha$ -adrenergic receptors. Moreover, the opiate receptor antagonist naltrexone remained fully effective in augmenting pulsatile LH secretion during concurrent phenoxybenzamine infusion.

As a complementary probe of adrenergic control of episodic gonadotropin secretion, we used  $\alpha$ -methyldopa, which is capable of depleting brain catecholamines in the rat (13, 14). This drug has also been shown to block progesterone-stimulated LH secretion in estrogen-primed menopausal women (3), but direct assessment of brain catecholamine depletion has not been possible in man. Using the exact dose and schedule (24-h pretreat-

ment) of  $\alpha$ -methyldopa employed in the latter work, our studies reveal no interruption of spontaneous pulsatile LH release in normal men. In addition, naltrexone's stimulation of increased episodic LH secretion was not impaired in the presence of this adrenergic inhibitor.

The present studies do not suggest that noradrenergic mechanisms play a critical role in maintaining basal or opiate-modulated pulsatile gonadotropin secretion in normal men. To our knowledge, these results provide the only data available regarding the actions of either phenoxybenzamine or  $\alpha$ -methyldopa on episodic LH release in normal men and represent the first test of the role of noradrenergic mechanisms in opioid-modulated pulsatile LH secretion in the human. Our results should be distinguished from other recent observations which have implicated dopamine receptors in opiate antagonist-stimulated LH secretion in man (15). Whether dopamine acts through brain or pituitary receptors under these circumstances has not been established.

The difference between the present results in normal men and previous reports in experimental animals may reflect species differences. Alternatively, since certain studies in the experimental animals were performed after oophorectomy, with or without sex steroid replacement, possible influences of gonadectomy or steroid hormones on noradrenergic mechanisms should also be considered. This consideration is important, because gonadal sex steroids are capable of modifying hypothalamic turnover of catecholamines and altering gonadotropin responsiv-

ity to opiate substances (10). Thus, results in normal men should not be applied facily to menstruating or menopausal women, in whom a different sex steroid milieu prevails.

The doses of phenoxybenzamine and  $\alpha$ -methyldopa used in the present work were chosen empirically, because of their previously demonstrated efficacy in suppressing LH release in the rhesus monkey and human (1, 3). In addition, choice of inhibitor doses on an empirical basis reflects the present inability to directly quantitate the degree of inhibition of brain noradrenergic transmission in the human. In man, one cannot do the invasive studies needed to confirm brain levels of noradrenergic blockade or catecholamine depletion, nor can one administer these drugs directly into the central nervous system to avoid the blood-brain barrier or employ the high toxic doses used in laboratory animals. Thus, it is possible that the doses of noradrenergic inhibitors used might not be capable of unmasking a role for brain noradrenergic neurons in basal or opiate-modulated pulsatile LH release in the human, despite effects of these inhibitor doses on LH release under other endocrine conditions in primate species.

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