Effects of valproate-induced alteration of the GABAergic system on pulsatile luteinizing hormone secretion in ovariectomized women

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It is well established that valproate increases hypothalamic concentrations of γ-aminobutyric acid (GABA). Although little research has been done on the role of GABA in the control of pulsatile luteinizing hormone (LH) secretion in humans, our group recently found that administration of valproate had no significant effect on pulsatile LH secretion in late follicular and mid-luteal phase normal women. However, the results of several studies of rats suggest that GABAergic regulation of LH secretion may depend on steroid levels. The objective of this work was to determine whether regular administration of sodium valproate inhibits pulsatile LH secretion in ovariectomized women. Twelve women who had undergone ovariectomy for causes other than malignant tumors were each studied in two 8 h sessions, in each of which blood samples were taken every 5 min. The first session was the control; for the second, 400 mg of sodium valproate was administered every 8 h during the seven preceding days and at 08.00 h and 14.00 h on the day of the study session. Serum valproate was determined by repolarization fluorescence spectrophotometry, and LH, estradiol and progesterone by radioimmunoassay. The serum LH series were subjected to a deconvolution procedure to reconstruct the pattern of pituitary LH secretion. Luteinizing hormone pulses were identified by the authors' non-parametric method. Control and post-valproate results were compared with regard to number of pulses, pulse duration, the quantity of LH secreted in each pulse, interpulse interval and mean serum LH level. There was no statistically significant difference between control and post-valproate results for any of the variables considered. It is concluded that sustained serum valproate levels do not alter pulsatile secretion of LH in ovariectomized women. This implies that, in humans, GABA is probably not a decisive factor in the regulation of the GnRH pulse generator.

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Anatomical and histological evidence that γ-aminobutyric acid (GABA) is involved in regulation of LH secretion has been provided by the location of a GABAergic tubero-infundibular neuron system (1–4), of GABAergic neurons that bear progesterone (5) and estrogen receptors (6) suggestive of involvement in feedback control of LH secretion (5, 7) and of GABAergic synapses on GnRH neurons (8, 9). However, studies in the rat have variously found that the effect of GABA on LH secretion is stimulatory (10, 11), inhibitory (12, 13) or both (14, 15).

It is known (16, 17) that GABA concentrations in the brain—especially in the hypothalamus (18)—are increased by sodium valproate. Specifically, valproate administration causes a rapid rise in GABA levels in presynaptic terminals (19) and an increased release of GABA into the synaptic cleft (20, 21). We reported recently that alteration of the GABAergic system by sodium valproate had no significant effect on pulsatile LH secretion by normal women during the late follicular phase (22) and mid-late luteal phase (23). However, the findings in rats that the rise in serum LH concentration following ovariectomy is associated with a fall in hypothalamic GABA levels, and that the fall in serum LH concentration following administration of estradiol is associated with an increase in hypothalamic GABA levels (7, 24), suggest that GABAergic regulation of LH secretion may depend on steroid levels (25, 26). If so, the absence of any significant change in LH secretion following valproate administration in our study of normal women may have been due to the high estradiol and progesterone levels of our subjects, which may have ensured that their basal GABA levels were already high enough to produce the maximum possible effect of GABA on LH secretion (27).

The aim of the study described here was to determine whether valproate-induced alteration of GABA levels does have an effect on LH secretion in ovariectomized...
women, in whom steroid levels are low. To avoid misunderstanding, we stress that we did not aim to evaluate either the hypothesis that steroid concentrations affect hypothalamic GABA levels or the effect of steroid levels on possible GABAergic regulation of LH secretion; our experimental design addresses neither of these issues directly. The former hypothesis merely suggested to us that ovariectomized women might constitute a group in whom any effect of GABA on LH secretion would be detectable by pharmacological manipulation of hypothalamic GABA levels.

Materials and methods

Subjects

We studied 12 32–46-year-old women 6 months (median) after ovariectomy for causes other than malignant tumors (leiomyoma, 6; endometriosis, 3; salpingo-oophoritis, 1; peritonitis and salpingitis, 1; ovarian cyst, 1) and before the start of hormone replacement treatment. The study was approved by the hospital ethics committee and all women gave informed written consent.

Methods

Each subject was studied in two sessions, 15–30 days apart, in which blood samples were taken every 5 min between 10.00 and 18.00 h. For the second but not for the first (control) session, 400 mg of sodium valproate (VPA: Depakine, Labaz) was administered every 8 h during the seven preceding days and at 08.00 and 14.00 h on the day of the study.

Luteinizing hormone was quantified in triplicate by RIA using a commercial kit (Famros Diagnostica, Finland). The standard deviation of replicate determinations with this kit depends on the LH level, and has been found during validation in our laboratory to be given by the expression (28)

$$SD = 0.04069 \times LH + 0.1169$$

Basal estradiol and progesterone levels were also determined by RIA, using kits from bioMérieux (France): the intra-assay coefficient of variation for estradiol was 6% and for progesterone was 8%. Serum VPA concentrations were determined by repolarization fluorescence spectrophotometry using the Abbott TDX system (sensitivity was 0.7 μg/ml and precision was better than 5%).

Signal analysis

Deconvolution method. To correct for the effect of clearance on serum LH levels (29) and so construct a more faithful picture of the time dependence of actual secretion, the LH data were subjected to a deconvolution procedure (30). Briefly, $x[k]$, the rate of LH secretion at time $kT$ (where $T$ is the sampling period), was calculated from previous $x$ values and serum LH values $y$ using the expression

$$x[k] = y[k] + a_0 y[k - 1] + a_1 y[k - 2] + a_2 x[k - 1]$$

which follows from $z$-transformation of the expression

$$y(t) = \int_0^t x(\theta)h(t - \theta)d\theta$$

when $h(t)$ corresponds to a two-compartment model of LH clearance

$$h(t) = f \exp(-0.693 t/t_1) + (1 - f) \exp(-0.693 t/t_2)$$

where $a_i$ in Equation (1) is a function of $t_1$, $t_2$ and $T$. In this work we used the values $t_1 = 18$ min, $t_2 = 90$ min and $f = 0.615$ (31).

Pulse identification. Luteinizing hormone pulse identification of the original LH signals and on the deconvolved series was performed by a method developed by our group (28) and based on Friedman’s non-parametric statistics. The variables extracted on the deconvolved LH signals were the number of pulses per 8 h, the area of each pulse (i.e. the quantity of LH secreted per pulse), the duration of each pulse and the interpulse interval; the variables analyzed on the original LH signals were the pulse amplitude (difference between cent and nadir) and the intercent interval. We also compared the mean serum LH concentration before and after VPA.

Statistics. To prove whether the analyzed variables followed a normal distribution, the Kolmogorov–Smirnov statistic was used (32); given that none of the variables were normal, the Mann–Whitney test was used. The mean serum LH and the number of pulses before and after VPA administration were compared with the Wilcoxon test.

Results

Serum VPA concentration after 7 days of administration was $513.56 \pm 170.80 \mu$mol/l (the therapeutic range is $345–690 \mu$mol/l (18)). No VPA was detected in blood samples taken during the control session. Sodium valproate administration was well tolerated by all subjects except one, who reported nausea and occasional vomiting after 5 days of administration and suffered two intense vomiting fits during the second study session. The date for this subject have not been included in the statistical analysis.

Serum estradiol concentration was $56.57 \pm 40.11$ pmol/l and the progesterone level was $0.28 \pm 0.14$ nmol/l. None of the LH secretion variables were statistically
Table 1. Mean ± s.d of the variables before and after valproate (VPA) treatment, with medians in parentheses.

<table>
<thead>
<tr>
<th></th>
<th>Pre-VPA</th>
<th>Post-VPA</th>
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<tr>
<td><strong>Pituitary pulses</strong></td>
<td></td>
<td></td>
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<tr>
<td>Area (IU/l)</td>
<td>18.34 ± 14.78 (13.69)</td>
<td>16.08 ± 15.80 (11.38)</td>
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<tr>
<td>Duration (min)</td>
<td>27.06 ± 15.53 (25)</td>
<td>24.81 ± 15.42 (20)</td>
</tr>
<tr>
<td>Interpulse interval</td>
<td>28.64 ± 16.56 (25)</td>
<td>27.38 ± 16.51 (25)</td>
</tr>
<tr>
<td>No. of pulses/8 h</td>
<td>15.00 ± 5.20 (11)</td>
<td>15.91 ± 4.70 (16)</td>
</tr>
<tr>
<td><strong>Serum LH pulses</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Amplitude (IU/l)</td>
<td>9.37 ± 7.08 (8.33)</td>
<td>10.64 ± 9.01 (7.77)</td>
</tr>
<tr>
<td>Intercent interval</td>
<td>39.66 ± 21.76 (35)</td>
<td>42.63 ± 2.75 (40)</td>
</tr>
<tr>
<td>No. of cents/8 h</td>
<td>10.18 ± 3.28 (11)</td>
<td>9.54 ± 2.98 (10)</td>
</tr>
<tr>
<td>Mean serum LH level</td>
<td>42.77 ± 13.37 (40.45)</td>
<td>40.15 ± 14.98 (39.74)</td>
</tr>
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*None of the differences between pre- and post-VPA values are statistically significant.*

significantly affected by VPA administration (Table 1). By way of illustration, Fig. 1 shows the serum LH profiles of two subjects with and without prior VPA administration, together with the corresponding deconvolved signals.

Discussion

The results of recent pharmacological studies of ovariectomized animals suggest that GABA plays a role in the regulation of LH secretion. Specifically, it has been reported that in these animals an increase in GABA concentration inhibits LH secretion, and that it does so by inhibiting the GnRH “pulse generator” (33) through the mediation of either GABA_B (34) or GABA_A (35) receptors. There is even evidence that increasing GABA concentration reduces GnRH gene expression (36) and GnRH receptor gene transcription in the preoptic area and the posterior mediobasal hypothalamus (37). Because GABA turnover in rat hypothalamus appears to be low in ovariectomized animals (and high in steroid-treated ovariectomized animal) (24), it seems possible that it is precisely this reduction in hypothalamic GABA that is largely responsible for the high LH secretion of ovariectomized rats (7).

There has been little direct research on whether GABA plays a role in the regulation of LH secretion in humans (38–41). In particular, hardly any work has been done on the possible relationship of the GABAergic system to the pulsatility of LH secretion (22, 23, 42). This low level of research activity has been due partly to the difficulty of reconstructing the dynamics of hypothalamo–pituitary interaction from peripheral...
blood samples, and partly to the lack of a broad group of safe, specific drugs allowing external control of this interaction. We have reported previously that VPA-induced alteration of the GABAergic system has no significant effect on pulsatile LH secretion by normal women during the late follicular phase (22) and mid-luteal phase (23) (and therefore no effect on mean serum LH concentrations during these periods), a finding in keeping with the results of other researchers (41–43).

The results on ovariectomized women now reported appear to confirm that, in this respect as in others, the neurotransmitter-mediated mechanisms controlling the hypothalamo–pituitary axis in humans differ from those operating in rats. Administration of VPA for 7 days neither significantly depressed the high LH levels caused by ovariectomy (44, 45) nor significantly affected the amplitude, frequency or duration of LH pulses. Because LH pulse frequency is a direct reflection of the frequency of “GnRH pulse generator” pulses (46), it may be concluded that VPA has no effect on the latter; and because GABA is known not to affect LH secretion by any direct action on the pituitary in vivo (10), our findings also show that VPA has no effect on the size of GnRH pulses either, because LH pulse amplitude, which exhibited no alteration in this study, depends on the rate at which GnRH reaches the pituitary (47), the frequency of GnRH pulses (which we have seen not to be altered significantly by VPA) (48) and steroid levels (which are constant in ovariectomized women) (49).

It seems unlikely that our negative results can have been due simply to VPA having had little effect on hypothalamic GABA concentration: although GABA was not determined in this study, it is well established that the VPA dosage used in this work increases GABA levels both in plasma (by 33–80% within 48–72 h (50–52)) and in various cerebral nuclei (17, 53), especially hypothalamus (by 95% (18)).

A more subtle issue is whether our negative results might not have been due to an LH-depressive GABA-mediated effect of VPA having been compensated for by some LH-increasing effect due to the action of VPA on other brain components. As far as we are aware, four brain components other than GABA have been reported to be affected by VPA: the serotonin metabolite 5-hydroxyindoleacetic acid (5-HIAA); the dopamine metabolite homovanillic acid (whose role in valproate is itself mediated by GABA (54)); neuron sodium channels; and aspartate. High 5-HIAA and low homovanillic acid were both observed in VPA-treated patients with schizophrenia and tardive dyskinesia (54); however, neither 5-HIAA nor homovanillic acid seems at all likely to have any effect on LH, because negative results have been obtained by all studies that have investigated whether pulsatile LH secretion in normal humans is affected by serotonin (55–58) or by anti-dopaminergics such as metoclopramide (59–61). Valproate-induced reduction of transmembrane sodium influx and high-frequency repetitive action potentials in in vitro neuron cultures has been related to the anticonvulsant activity of VPA (62), but there has been no research on the implications of these in vitro effects for GnRH secretion; it may be pointed out that any hypothetical reduction in the activity of the GnRH pulse generator due to this cause would tend to reinforce rather than compensate for a depressive effect of GABA.

Finally, VPA has been found to reduce brain aspartate levels (18) and the stimulant effects of N-methyl-D-aspartate (NMDA) (63) in rat neocortex. These effects are indeed more relevant to LH secretion than those discussed above, because NMDA reduces the amplitude of LH pulses in ovariectomized rhesus monkeys (64); but there is no evidence that a reduction in aspartate levels or in response to aspartate has the opposite effect of increasing LH pulse amplitude, or that it modifies LH pulse frequency.

In this study, LH secretion was unaltered after high GABA levels had presumably been maintained for 1 week by therapeutic doses of VPA. By contrast, two research groups have found that transient elevation of GABA levels or of the activation of GABA receptors is associated with alteration of LH secretion. Spremovic-Radjenovic et al. (65) have found that a pronounced but transient inhibition of LH secretion occurs a few hours after administration of a single therapeutic dose of VPA; and Judd et al. (66) have found that a single dose of alprazolam—a benzodiazepine derivative that stimulates the GABA_A receptors—in the follicular phase reduces the frequency and increases the amplitude of the LH pulses. It nevertheless seems possible that the VPA-induced depression of LH secretion observed by Spremovic-Radjenovic et al. may not have been mediated by GABA, because this depression of LH secretion is analogous to a similarly transient depressive effect of VPA on the spontaneous spike-wave syndrome in rats (67), an effect that cannot be mediated by GABA because GABA aggravates the spike-wave syndrome. Interpretation of Judd et al.’s findings, on the other hand, is hindered by their having used alprazolam, which acts only at GABA_A receptors and will therefore not have had all the effects that GABA has; in any case, it remains to be seen whether the observed effect of transient elevation of GABA_A activation is really indicative of control of the GnRH pulse generator or instead reflects merely a temporary imbalance with no regulatory significance.

Finally, we point out that the results obtained in this study by means of a deconvolution technique confirm that, as in the cases of men (68) and late follicular phase women (69), the quantity of LH secreted in each pulse is considerably underestimated by conventional methods of studying pulsatile secretion. As regards the frequency of pituitary LH pulses, interpulse intervals of about 30 min measured in this study were shorter than those reported previously for post-menopause women (44) but their accuracy is supported by the similarity...
between the serum LH pulse amplitudes measured in this work and the mean value of 9 IU/L observed by Crowley and co-workers (70) when they administered GnRH at 30-min intervals to patients with hypogonadotropic hypogonadism.

In conclusion, in this study we found that for ovariectomized women, as for normal women in the follicular and luteal phases, sustained administration of VPA has no significant effect on pulsatile LH secretion. We conclude that sustained elevation of hypothalamic GABA concentration does not inhibit LH secretion in women, and that GABA therefore has no preponderant role in the control of the GnRH pulse generator in women.

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