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A Comparison of γ-Butyrolactone and Pimozide on Serum Gonadotrophins and Ovulation in the Rat (39498)

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Hydrolytic cleavage of γ-butyrolactone (GBL) *in vivo* produces the unique CNS depressant, γ-hydroxybutyrate (GHB) (1). While both compounds produce CNS depression, this latter compound is exceptional in that it is a normal brain metabolite (2) and produces a significant increase in brain dopamine (DA) without affecting other brain neurotransmitters such as norepinephrine (NE), serotonin (5HT), or γ-aminobutyric acid (GABA) (3-6).

In view of the postulated role the dopaminergic tuberoinfundibular system plays in gonadotrophin release and subsequent ovulation (7-10), we have compared the effects of GBL and pimozide, a fairly specific dopaminergic receptor blocker, on the proestrous gonadotrophin surge and ovulation. While GHB passes the blood-brain barrier and appears to be the active moiety (1, 11, 12), we have employed GBL since it is immediately hydrolyzed to GHB in plasma and liver and is more rapidly and uniformly taken up and distributed than GHB after intraperitoneal injection (1). Although high doses of pimozide previously have been reported to delay ovulation and reduce the proestrous LH surge (13), no dose correlation was made between an anti-ovulatory effect and the proestrous gonadotrophin surge.

Materials and methods. Mature female Sprague-Dawley (S-D) rats (Charles River, Cambridge, Massachusetts), 225-250 g in weight and acclimated to laboratory conditions, were maintained on a fixed 14-hr light/10-hr dark lighting schedule (lights off 1900 hr). Only those rats exhibiting at least two consecutive 4-day cycles were used. γ-Butyrolactone (n_D^{20} 1.4365, Aldrich Chemical Co.) was diluted with saline and injected ip at 1330 hr on proestrus. Pimozide (1(1-[4,4,-bis(*p*-fluorophenyl)butyl]-4-piperidyl)-2-benzimidazolinone; Janssen Pharmaceutica) in corn oil was injected sc at 1200 hr proestrus. Sequential blood samples for determination of serum LH and FSH by RIA (14) were taken by substernal heart puncture (0.5-1.0 ml; volume replaced ip by saline) under light ether anesthesia hourly from 1330-1730 hr proestrus. In our hands, cardiac puncture, via substernal entry, does not overcome pentobarbital's blockade of ovulation (15), nor is there a significant difference in serum LH and FSH between rats with indwelling cannulae (16) and cardiac puncture (C. W. Beattie, unpublished observations). All values for serum LH and FSH are reported irrespective of whether the animal ovulated. Autopsies were performed on the morning of expected estrus and the degree of ovulation was assessed by counting tubal ova.

Serum LH and FSH levels were determined by RIA from respective kits supplied by the NIAMDD Rat Pituitary Hormone Program and by Dr. A. Parlow. LH was assayed from duplicate 25-μl samples. FSH was determined in duplicate 50-μl samples. The lower limit of sensitivity for both hormones was 10 ng/ml. Values were expressed in terms of LH and FSH RP-1, respectively.

Results. The degree of ovulatory inhibition produced by increasing doses of GBL is illustrated in Table I. The antiovulatory ED_{50} was approximately 250 mg/kg, which is a subanesthetic dose. At this dose, increases in uterine wet weight (luminal fluid expressed) accompanied the increased incidence of uterine ballooning, but only the 750-mg/kg dose of GBL produced a significant increase over control. No change was noted in ovarian weight.

Proestrous serum LH levels from the rats described in Table I are illustrated in Fig. 1. GBL produced a significant dose-related decrease in serum LH levels over the time period sampled. By 1630 hr, proestrous FSH levels (Fig. 2) were significantly reduced by doses of GBL above the anti-ovulatory ED_{50}.

TABLE 1. INHIBITION OF OVULATION WITH γ-BUTYROLACTONE (GBL) OR PIMOZIDE.

Drug	mg/kg	N	Number of rats ovulating	Percentage inhibition of ovulation	Number of ova in ovulating rats
Saline	–	15	15	0	13.2 ± 0.5+
GBL (ip)	750*	5	0	100	0
	500*	7	2	71	13, 15
	250	11	4	63	11.7 ± 0.9
	125	5	4	20	9.5 ± 1.6
	62.5	9	7	22	12.3 ± 0.7
Oil (sc)	–	5	5	0	12.6 ± 0.9
Pimozide (sc)	2.5	6	0	100	0
	1.25	6	2	67	11, 12
	0.75	5	3	40	7, 13, 8
	0.63	4	4	0	10.0 ± 1.0

+ Mean ± standard error.
* Anesthetic dose.

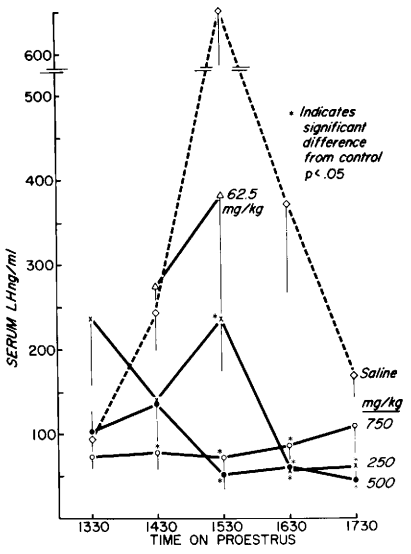

FIG. 1. The effects of γ-butyrolactone (ip) on the proestrous serum LH surge. Each point represents five to eleven animals. Values are expressed as mean ±SE.

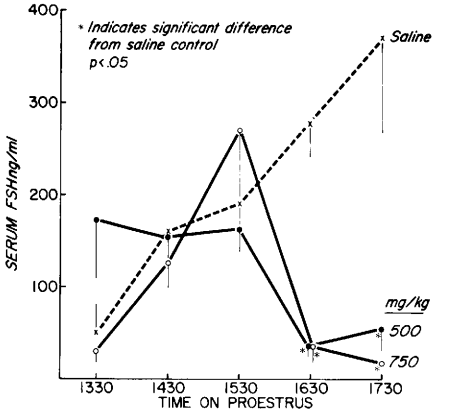

FIG. 2. The effect of γ-butyrolactone on the proestrous serum FSH surge. Each point represents five to eleven animals. Values are expressed as mean ±SE.

Pimozide significantly reduced proestrous serum FSH and LH values (Fig. 3) over the sampling period at its approximate antiovulatory ED_{50} doses (Table I).

Discussion. These data demonstrate for the first time that GBL blocks ovulation. Since GHB appears to be the active moiety (1, 11, 12), it is likely to be responsible for the suppression of the proestrous gonadotrophin surge and subsequent inhibition of ovulation. While the exact mechanisms and sites of the GBL blockade of ovulation are unknown, it appears that GBL, like pimozide, inhibits ovulation by suppressing the CNS-induced proestrous gonadotrophin surge.

The neuropharmacology of GBL differs markedly from pimozide. GBL or GHB produces an increase in brain DA content which is accompanied by a reduction in impulse flow within central dopaminergic neurons (17), coupled with a decrease in DA release and/or metabolism (6). In this respect, it differs from pimozide, which selectively decreases brain DA concentration at low doses (18). Clearly, an alteration in brain DA content per se is not directly re-

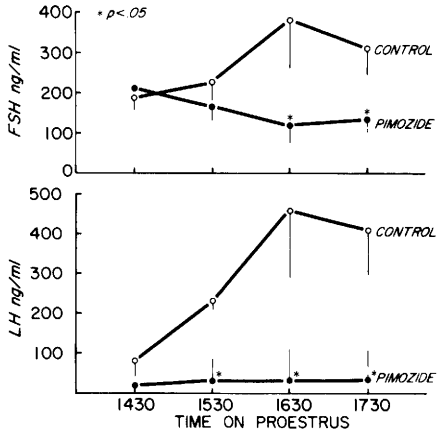

FIG. 3. The effect of pimozide (1.25 mg/kg sc) on serum LH and FSH levels of proestrous rats. Each point represents four to six animals. Values are expressed as mean ±SE.

lated to ovulation inhibition or to a decrease in the proestrous surge of gonadotrophins.

If one considers only the turnover of central DA as it affects gonadotrophin release and ovulation, one finds that GHB appears to decrease the turnover of DA (5, 6), while pimozide increases DA turnover (18). It appears, therefore, that neither DA content nor alterations in turnover per se affect proestrous gonadotrophin release and subsequent ovulation. The single thread of continuity after administration of either of these agents affecting dopaminergic impulse flow in the intact rat appears to be that any interruption, pre- or postsynaptically, of central dopaminergic nerve impulse flow over a discrete time period, alters gonadotrophin release and subsequent ovulation.

It is noteworthy that pimozide only began to decrease proestrous serum gonadotrophin levels and inhibit ovulation at doses that impinge upon central NE content and/or turnover (18). Barbiturates also begin to affect central (cortical) NE function (19) at anti-ovulatory doses (20).

Collectively, the present studies suggest that in the intact proestrous rat any interruption of central dopaminergic impulse flow significantly decreases LH and FSH secretion and inhibits ovulation. These results also suggest that in the intact animal, high doses of putative dopaminergic blockers, which affect noradrenergic as well as dopaminergic neurons, are necessary to significantly reduce the proestrous gonadotrophin surge and subsequently ovulation.

Summary. Proestrous serum LH and FSH levels and ovulation were significantly reduced when either γ-butyrolactone (GBL), which is hydrolyzed *in vivo* to the naturally occurring CNS depressant γ-hydroxybutyrate (GBH), or pimozide, a dopaminergic receptor blocker, was administered to 4-day cyclic rats just prior to the proestrous critical period. These data suggest that GBL and pimozide inhibit ovulation by decreasing the proestrous serum LH and FSH surges through effects on a central dopaminergic pathway.

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