CNS Depression in the Absence of Prolactin Release Following Intraarterial Injections of Sodium Pentothal in the Proestrous Rat[1] (37726)

JOHN H. WEDIG[2] AND VERNON L. GAY[3]
(Introduced by A. R. Midgley, Jr.)
Interdepartmental Toxicology Program and Reproductive Endocrinology Program, Department of Pathology, University of Michigan, Ann Arbor, Michigan 48104

In the rat, a preovulatory increase in serum luteinizing hormone (LH), follicle stimulating hormone (FSH), and prolactin occurs spontaneously on the afternoon of proestrus (1–5). Although these three hormones are released at approximately the same time of day, available data indicate that separable mechanisms may exist for the release of individual hormones. For example, pentobarbital anesthesia virtually abolishes the preovulatory "surge" of LH release but does not prevent the increase in serum FSH measured on the morning of estrus (5). We have presented evidence to suggest that pentobarbital does not prevent LH secretion by a direct inhibition of the pituitary gland (6), as well as evidence to suggest that such anesthesia does inhibit L-Dopa-induced LH release in the proestrous rat (7).

The effects of pentobarbital anesthesia on prolactin release may be quite complex, since such anesthesia produces a prompt increase in serum prolactin in the proestrous rat but inhibits subsequent spontaneous preovulatory release (2). The mechanisms by which pentobarbital and ether (1, 4, 8) anesthesia initially stimulate prolactin release have not been defined, but may be related to the increase in serum prolactin which is evoked by stress in both male and female rats (4, 9, 10). Although it appears that the inhibitory effects of pentobarbital on preovulatory prolactin release may be due to a direct action on the pituitary gland (11), the initial increase in serum prolactin during the first hour following anesthesia is generally thought to result from altered secretion of hypothalamic regulatory factors (11, 12). However, currently available data provide no distinction between decreased secretion of prolactin-inhibiting factor (PIF) or increased secretion of prolactin-releasing factor (PRF) as the mechanism responsible for prolactin release under these conditions.

If increased prolactin secretion results from a decrease in hypothalamic activity and PIF secretion, it would appear that any and all anesthetic agents which cause a depression of CNS activity might also cause an increase in prolactin release. However, we present data in this report to indicate that another anesthetic agent (sodium pentothal) induces anesthesia without concomitant release of prolactin. These data suggest that decreased CNS activity *per se* need not lead to increased prolactin secretion.

Materials and Methods. Mature female rats (240–260 g) of the Holtzman strain were housed in air-conditioned quarters under controlled lighting (lights on 5 AM to 7 PM) for 10 days. Vaginal smears were obtained daily at 9 AM for an additional 2 weeks before beginning the experiments. Rats with either 4- or 5-day cycles were used. At 9

[1] Supported by U.S. Public Health Service Grants HD-05318 and ES-00106. Address reprint requests to: Dept. of Pathology, University of Michigan, Ann Arbor, MI 48108.

[2] Recipient of an NIH Toxicology Training Fellowship. Present address: Olin Research Center, 275 Winchester Avenue, New Haven, CT 06504.

[3] Career Development Awardee of the National Institute of Child Health and Human Development. Present address: Dept. of Physiology, University of Pittsburgh School of Medicine, Pittsburgh, PA 15261.

AM on the day of functional proestrus (determined by vaginal cytology and confirmed by the presence of uterine fluid), the rats were anesthetized with ether, cannulated via the carotid artery (13), and used approximately 4 hr later. At the beginning of the experiment, 100 U of heparin in a volume of 100 μl was injected via the cannula. Prior to obtaining each blood sample, 40 μl of blood was withdrawn and discarded. The cannula was flushed with 40 μl of warm physiological saline immediately after obtaining each blood sample and after infusion of anesthetics. Each 50-μl sample of whole blood was added directly to an assay tube as described previously (13). Blood samples were stored at 4° for 1–3 days before being assayed for LH (14) or prolactin (15) by double-antibody radioimmunoassay systems. Serum prolactin concentrations have been expressed in terms of B329, a highly purified rat anterior pituitary gland extract, 1 mg of which is equivalent in biological activity to 30 IU of prolactin. Serum luteinizing hormone concentrations have been expressed in terms of B160, a partially purified rat anterior pituitary gland extract, 1 mg of which is equivalent to 0.17 mg of NIH-LH-S1 as determined by bioassay.

Nineteen proestrous rats were injected with sodium pentobarbital (40 mg/kg, ip) at 1:20 PM while 4 similar rats received intraarterial injections (25 mg/kg) at 11:00 AM. A group of 14 proestrous rats received three intraarterial injections of sodium pentothal (20 mg/kg/injection) via the cannula over 2.5-min intervals beginning at 1:00, 1:15, and 3:00 PM. Control animals were cannulated on the morning of proestrus but received only an ip injection (0.2 cm^3) of warm physiologic saline at 1:20 PM. In all groups, sequential blood samples were obtained 20, 10, and 1 min prior to the initial barbiturate or saline injection and at 20-min intervals thereafter until 5:40 PM. The following morning (day of estrus) all rats were sacrified, and their oviducts and uteri were examined for the presence of tubal ova or accumulated uterine fluid.

Results. In initial (preinjection) blood samples, plasma prolactin levels in all groups ranged from 10 to 20 ng/ml and were comparable to serum concentrations reported for unanesthetized female rats (9). In nine control proestrous rats, cannulation and collection of sequential blood samples did not interfere with gonadotrophin release, ovulation, or the subsequent appearance of tubal ova. Anesthesia, maintained during the critical period (2–4 PM) on the afternoon of proestrous by a single ip injection of pentobarbital or by multiple intraarterial injections of pentothal, prevented LH release (Fig. 1) and blocked ovulation. An intraarterial injection of pentobarbital at 11:00 AM or an ip injection at 1:20 PM resulted in a rapid increase in serum prolactin, followed by a return to basal levels approximately 120 min later (Figs. 1 and 2). Injections of saline into control proestrous animals did not cause this immediate release of prolactin nor did multiple injections of pentothal (which blocked ovulation) cause an increase in serum prolactin (Fig. 1). Thus, pentobarbital induced an immediate increase in serum prolactin but prevented subsequent release. Pentothal also blocked spontaneous prolactin release, but did so without inducing an initial increase in serum prolactin concentrations.

In two separate experiments (4 rats/group), pentothal anesthesia at 12:55 PM or 1:15 PM on the day of proestrus prevented an increase in serum prolactin following subsequent ip injections of pentobarbital at 1:35 PM or 3:00 PM, respectively.

Discussion. In the rat, pentobarbital anesthesia prevents the spontaneous preovulatory release of both LH and prolactin, but causes a rapid and transitory release of prolactin at the time of anesthesia. Since a considerable body of evidence is available to suggest that the brain normally inhibits prolactin secretion (12), it has been suggested that pentobarbital anesthesia may interrupt this tonic inhibition (11). More recently, evidence has been presented to suggest that the hypothalamus of the rat contains a prolactin-releasing factor (PRF) which acts to stimulate prolactin release (16) but is distinct from the tripepetide which stimulates TSH and prolactin release in the

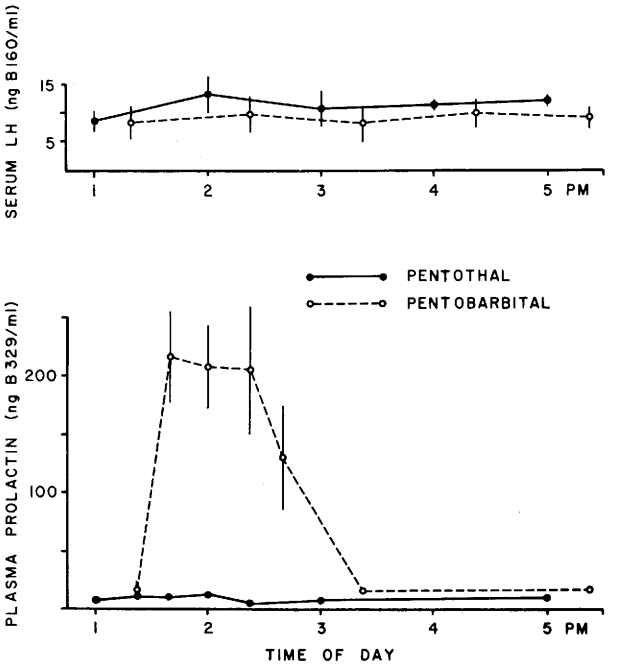

FIG. 1. Serum prolactin and LH concentrations (± SE) in sequential blood samples collected from cannulated proestrous rats receiving either an ip injection of pentobarbital (40 mg/kg) at 1:20 PM or a series of three intraarterial injections of sodium pentothal (20 mg/kg/injection) beginning at 1:00 PM (8 rats/group). Points without estimates of standard error were below the sensitivity of the assay.

rat and human (17–19). Although the data presented here permit no distinction between decreased secretion of PIF or increased secretion of PRF as the stimulus for pentobarbital-induced prolactin release, they do demonstrate that CNS depression can be induced in the absence of prolactin release. Such a demonstration indicates that a depression of hypothalamic secretion need not be considered a preferential explanation for this type of prolactin release.

Various stressful stimuli also produce a rapid increase in serum prolactin concentration, suggesting that the excitory phase of anesthesia may provide the initial stimulus for prolactin release (9, 10). The ability of sodium pentothal to induce an anesthetic state without prolactin release may therefore be associated with the rapid action of the intraarterially administered anesthetic on either the hypothalamus or the pituitary gland. However, pentothal appears to be relatively unique in this respect, since our data on the intraarterial injection of pentobarbital (Fig. 2) indicate that this route of administration does not prevent the stimulation of prolactin release.

Because of the limited quantity (25 μl) of serum in each assay tube and the lack of duplication for each point, the measurements of basal serum LH and prolactin may be less precise than similar values reported previously from this laboratory (15). For this reason, we attach little significance to the quantitative aspects of the minimum plasma concentrations reported here. However, these technical limitations do not apply to the plasma prolactin values in the range of 100 to 300 ng/ml, since samples of plasma containing such concentrations were measured with considerable precision and in the optimal portion of the standard curve.

Although these studies do not provide a clarification of the mechanisms underlying anesthesia induced prolactin release, they do provide a separation of two previously corre-

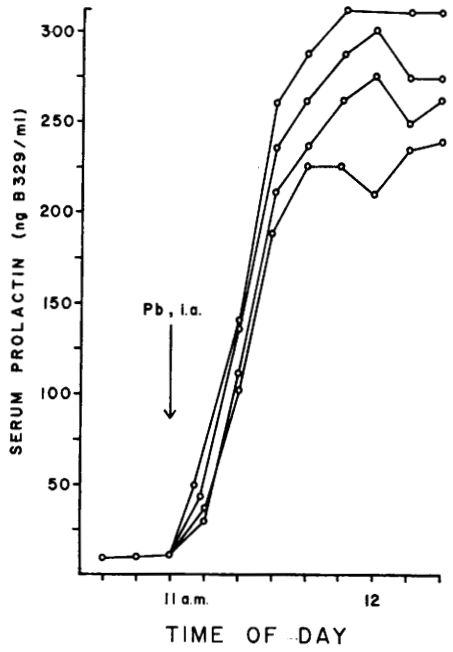

FIG. 2. Serum prolactin concentrations in four proestrous rats prior to and following the intra-arterial injection of pentobarbital (25 mg/kg) at 11:00 AM.

lated sequelae of anesthesia. This distinction would appear to enhance the prospects of defining both the stimulatory and inhibitory actions of anesthetics in this system.

Summary. Samples of whole blood were collected at 20-min intervals for a period of several hours from individual cannulated proestrous rat. Plasma luteinizing hormone and prolactin concentrations were determined by radioimmunoassay. Either pentobarbital or pentothal anesthesia maintained during the critical period in proestrus rats blocked the preovulatory increase in serum LH and prevented subsequent ovulation. Pentobarbital anesthesia induced a rapid increase in serum prolactin concentrations whereas pentothal anesthesia did not, but both anesthetics prevented the spontaneous preovulatory increase in serum prolactin. In addition, pentothal anesthesia prevented the response to subsequent injections of pentobarbital.

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