The Effect of Pentobarbital (Nembutal) on Prolactin Release In the Rat (36742)

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The factors controlling prolactin release in the rat are complex and incompletely understood; however, it has become clear that prolactin release is labile and can be increased by such diverse stimuli as suckling (1), stress (2, 3), estrogen (4, 5) and various drugs (6, 7). Recently, dopamine has been shown to be capable of inhibiting prolactin release both *in vivo* and *in vitro* (8–10). Conversely, drugs which lower hypothalamic dopamine stores have been found to elevate plasma prolactin (7).

Pentobarbital (Nembutal) has been previously shown to block the rise in blood prolactin during proestrus (11, 12) and following the stress of ether (3). The present paper deals with the effect of Nembutal on prolactin release in response to several diverse stimuli.

Methods. Female Sprague-Dawley rats (220–250 g body wt) were ovariectomized 4–5 wk before use. They were housed in a room maintained at 24° which was illuminated for 14 hr/day (lights on 5 AM to 7 PM) and given free access to water and laboratory chow. All blood samples were taken from the jugular vein while the rat was anesthetized with ether. Blood collection was completed after 2 min of exposure to ether. Plasma prolactin levels were determined using the radioimmunoassay kits supplied by NIAMD.[2] The results were expressed in terms of the standards supplied with the kits.

In the first experiment, the effect of Nembutal on the plasma prolactin concentration in ether stressed rats was examined. Rats were bled repeatedly while etherized at 10, 20, 30, 40, 60 and 120 min after intraperitoneal (ip) injection of Nembutal (35 mg/kg).

In the second experiment, *dl*-α-methyl-*m*-tyrosine (αMMT), an inhibitor of catecholamine synthesis, was injected ip at a dose of 200 mg/kg. In the third experiment, estradiol benzoate (EB) (5 μg/rat) was given sc.

Results. In the rats of the first experiment, to which were all bled while anesthetized with ether, prolactin levels were elevated as observed earlier (3). A marked drop in prolactin levels was noted within 10 min after Nembutal injection (Fig. 1). The level decreased further at 20 min and remained low for the 2 hr duration of the experiment. On the contrary, control animals which were injected with an equal volume (0.2 ml) of 0.9% NaCl solution ip and bled under ether anesthesia had elevated prolactin levels at all time intervals studied in comparison with controls which were decapitated and bled (not shown). There was a small but significant rise in plasma prolactin levels at 10 and 20 min after the first bleeding. The values then declined at 30 min to preinjection level and remained at fairly constant levels for 2 hr.

In the second experiment, *dl*-α-methyl-*m*-tyrosine (α-MMT), an inhibitor of catecholamine synthesis, was administered intraperitoneally at a dose of 200 mg/kg. One hour after the injection of α-MMT, the rats were injected with Nembutal (35 mg/kg, ip) immediately after collection of the second blood sample. As shown in Fig. 2, a pronounced elevation in plasma prolactin level was present 1 hr after the α-MMT treatment. This high level of prolactin was reduced to the preinjection level within 1 hr after Nembutal treatment and then remained low dur-

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[2] Kindly supplied by NIAMD.

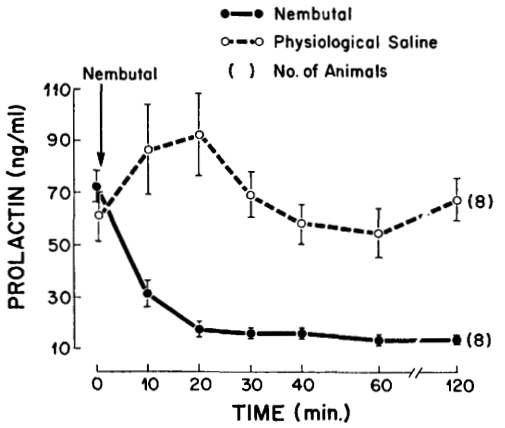

FIG. 1. Ability of Nembutal to lower plasma prolactin in ovariectomized rats bled while anesthetized with ether. In Figs. 1–3 (●) or (○) the mean and the vertical bars ± one standard error of the mean.

ing the remainder of the experiment. In control animals which were injected with α-MMT alone, the plasma level of prolactin remained high for 2 hr and then declined somewhat at 3 to 4 hr after α-MMT.

In the third experiment, a 6-fold elevation in plasma prolactin level was observed 48 hr after the subcutaneous injection of estradiol benzoate (EB) (5 μg/rat) (Fig. 3). Intraperitoneal injection of Nembutal (35 mg/kg) reduced this high level of prolactin to the level observed prior to estrogen within 1 hr. This level of prolactin then gradually increased 3 and 5 hr after the Nembutal injection and had returned to the high level observed after EB treatment by 24 hr. On the other hand, the control animals which were injected ip with physiological saline exhibited much smaller but significant reductions in plasma prolactin concentration after 1 and 3 hr with a rebound observed at 5 and 24 hr after the injection to levels similar to those observed prior to the injection of saline.

Discussion. The present results provide additional evidence of the ability of Nembutal to suppress prolactin release. Since Nembutal does not block the response of the pituitary to CRF (13) or LRF (3), we believe that its actions at the doses used are exerted on the central nervous system rather than on the pituitary gland itself.

In our previous experiments (3) using a slightly lower dose of Nembutal (31.5 mg/kg) some rats showed an initial discharge of prolactin following injection of Nembutal and Wuttke and Meites (12) described a release of prolactin following this dose of Nembutal in the proestrous rat. We believe that the release observed in our previous study was caused by the lower dose of Nembutal which was not sufficient to suppress the release of prolactin from the stress of ether plus bleeding. The data are consistent with the hypothesis that stress activates prolactin release via the hypothalamus. In view of the rapidity of the stress-induced rise (2 min to triple plasma levels) (3), we have speculated that it may be brought about at least in part by a prolactin-releasing factor (PRF) since the rate of rise if prolactin-inhibiting factor (PIF) were no longer secreted would be anticipated to be less. Nembutal apparently stops the release of PRF leading to a rapid fall in prolactin.

As expected, injection of the inhibitor of catecholamine synthesis, α-MMT, led to a dramatic increase in prolactin (6). This was thought to be caused by reduction of adrenergic stimulation of PIF release (7), thus relieving prolactin from inhibitory hypothalamic control. Surprisingly, the CNS depressant Nembutal, completely reversed the

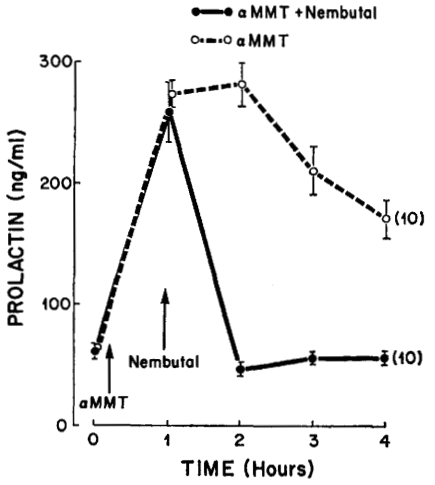

FIG. 2. Effect of Nembutal on the elevation of plasma prolactin producted by α-methyl-*m*-tyrosine (α-MMT).

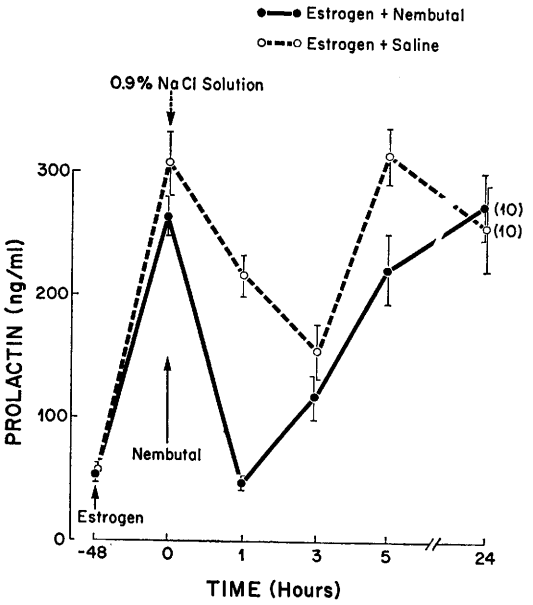

FIG. 3. Effect of Nembutal on the elevation of plasma prolactin produced by estradiol benzoate (5 μg).

rise and led to a fall of prolactin below the initial values. A possible explanation of this paradoxical result would be to postulate once again a PRF and to place it under inhibitory adrenergic control. α-MMT would thus lead to a stimulation of PRF discharge which would again be inhibited by Nembutal.

Estrogen stimulated prolactin release markedly as reported earlier (4, 5), and this release was also blocked by Nembutal. It would be reasonable to speculate that at least part of the action of estrogen is via increased release of PRF which was again blocked by Nembutal.

Alternate explanations of the data would involve a direct suppression of the pituitary by Nembutal which we feel to be unlikely as indicated above or the possibility that Nembutal selectively stimulates PIF discharge. This would appear unlikely since it suppresses release of other hypothalamic factors (3, 11, 13).

Summary. The effect of Nembutal on plasma prolactin levels of ovariectomized rats was investigated in three situations which bring about increased release of the hormone. Prolactin was determined by radioimmunoassay. Nembutal lowered the elevated plasma prolactin in etherized rats. It completely reversed the elevation induced by α-methyl-*m*-tyrosine, an inhibitor of catecholamine synthesis, and also reduced the elevated levels observed in estrogen-treated animals. The results support the hypothesis that Nembutal blocks the release of a prolactin-releasing factor which is released by ether stress, α-methyl-*m*-tyrosine and estrogen.

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