The Influence of Neural Blocking Agents Injected into the Third Ventricle of the Rat Brain and Hypothalamic Electrical Stimulation on Serum Prolactin[1] (36505)

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It has been established in the last decade that the secretion of anterior pituitary (AP) hormones is controlled by factors found in the hypothalamus and specifically, the median eminence. More recent interest has been directed to the influence of biogenic amines on AP hormone regulation. Fuxe and Hökfelt (1) reported that changes in catecholamines could be observed when the endocrine state of the animal was altered. The administration of a number of drugs that are active in altering neural biogenic amines has resulted in increases and decreases in AP hormones (2). Prolactin secretion, which is regulated by a hypothalamic inhibitory factor (PIF), has been increased by the administration of reserpine (3, 4), chlorpromazine (5, 6) and other neural drugs that nonspecifically alter biogenic amine activity (7). Recent work using drugs specific for catecholamines indicates that dopamine may play a role in prolactin regulation (8, 9). Kamberi, Mical and Porter (10) has presented evidence that dopamine and not norepinephrine suppresses prolactin secretion and that the action of dopamine is not directly on the AP but probably by stimulating the release of PIF. The catecholamine–prolactin relationship has been actively studied but little attention has been given to the influence of cholinergic elements on prolactin secretion. We would like to report the results of the injection of neural blocking agents into the third ventricle of the brain with and without the electrical stimulation of the ventral medial hypothalamus on serum prolactin levels as measured by radioimmunoassay (RIA).

Materials and Methods. Female Charles River C-D rats were vaginally smeared daily to obtain estrous cycle histories. Animals exhibiting 3 or more normal 4-day cycles were included in the study; their body weights were 250–300 g. On the day of vaginal estrus the animals were anesthetized with chloral hydrate (36 mg/100 g body wt ip) and a cannula (PE 50) was inserted into the tail artery to obtain blood samples. The animals were then placed onto a stereotaxic instrument and a 28 gauge stainless steel cannula was introduced into the third ventricle of the brain. Parallel electrodes were constructed from 0.01 in. diameter stainless steel enameled wire (Nilstain No. 304) and coated with epoxylite. The positive pole was 1 mm longer than the negative pole and approximately 1 mm of insulation was removed from the tip of each pole. The electrodes were unilaterally implanted into the ventral medial hypothalamus (VMH). The stimulus consisted of a rectangular biphasic pulse with an intensity of 200 μA, a duration of 1 msec, a frequency of 100 cps and an on–off time of 30 sec. The duration of stimulation was for 30 min. Atropine sulfate (Calbiochem) was injected at a level of 25 μg/5 μl into the third ventricle and at a level of 35 mg/kg body weight subcutaneously. Propranolol (Inderal, Ayerst Labs.) and phenoxybenzamine (Dibenzyline, Smith Kline & French Labs.) were injected at a level of 50 μg/5 μl into the ventricle. Control injections

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consisted of 5 μl of either saline or acid saline (pH 3.0). Since no difference was observed between the two saline groups, the data were pooled into a single sham control group. The experimental procedure consisted of an intraventricular or sc injection followed 5 min later by the initial or "before" blood sample (0.3 ml). Immediately after collection of initial blood sample electrical stimulation was initiated for 30 min. Blood samples were collected 30, 45, 75 and 135 min after the initiation of electrical stimulation. The location of the electrode was marked by a lesion using a 1 mA dc current for 30 sec. Cannula placement was marked by injecting 5 μl of India ink. The brains were fixed in formalin–acid–alcohol solution and sectioned to determine the proper placement of electrode and cannula. Any animal not having proper placement was discarded from the results. The curves represent the data from 3 to 5 animals/group. Serum prolactin levels were determined by rat prolactin radioimmunoassay which has been previously presented (11) and will be subsequently published in detail. The results are presented as differences from the initial value because of the marked variability in serum prolactin levels. The variability was probably due to collecting samples at various times throughout the day of vaginal estrus, a time when serum prolactin level changes markedly. Statistical analysis of the data was

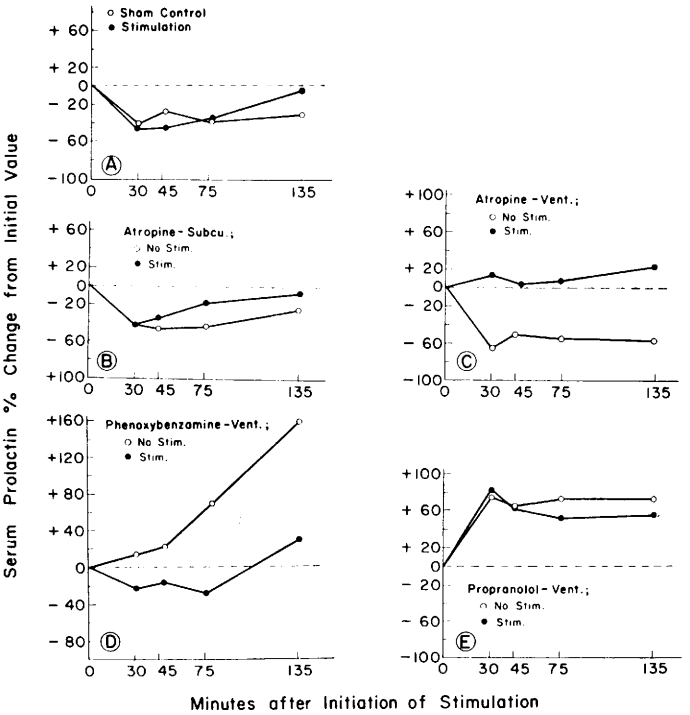

FIG. 1. Percentage change in serum prolactin concentration from initial (prestimulation) value. Cannula was in the third ventricle of the brain. Unilateral electrode was implanted in the ventral medial hypothalamus (VMH). (A) Influence of 5 μl of saline or acid saline (sham controls) alone and with electrical stimulation of VMH (see text for parameters). (B) Influence of 35 mg/kg body weight of atropine injected sc alone and with electrical stimulation of VMH. (C) Influence of 25 μg/5 μl of atropine injected into the third ventricle alone and with electrical stimulation of the VMH. (D) Influence of 50 μg/5 μl of phenoxybenzamine injected into the third ventricle alone and with electrical stimulation of the VMH. (E) Influence of 50 μg/5 μl of propanolol injected into the third ventricle alone and with electrical stimulation of the VMH.

initially performed by analysis of variance for two-way classification as described by Dixon and Massey (12) with experimental treatment as one classification and sample time as the other. Since there was no significant difference in sample time and interaction, the data were combined and analyzed by a one-way analysis of variance with the difference among treatment means assessed by Kramer's modification (13) of Duncan's multiple range test (14). The latter test is a conservative one for statistical significance.

Results. Serum prolactin levels decreased 30–40% in sham control animals (Fig. 1A). Electrical stimulation of the VMH after the injection of 5 μl saline into the ventricle resulted in a decrease in prolactin secretion similar to that of sham controls ($p > .05$). The subcutaneous administration of atropine, with and without electrical stimulation (Fig. 1B), did not alter the percentage decrease in serum prolactin from that of the respective controls ($p > .05$). Intraventricular injection of atropine decreased serum prolactin levels, while electrical stimulation increased serum prolactin levels from that of their respective controls (Fig. 1C). The differences, however, were not significant ($p > .05$). The 60% difference in serum prolactin levels between stimulated and nonstimulated animals receiving intraventricular atropine was found to be statistically significant ($p < .06$). Phenoxybenzamine an α-adrenergic blocker, resulted in a significant ($p < .01$) increase in serum prolactin when compared with saline controls (Fig. 1D). Electrical stimulation of VMH abolished the effect of phenoxybenzamine resulting in serum prolactin levels similar to that of electrical stimulation alone (Fig. 1D vs A). The injection of propranolol, a β-adrenergic blocker, with and without electrical stimulation, resulted in a 100–110% increase in serum prolactin levels when compared with respective controls (Fig. 1E). The differences were highly significant ($p < .01$).

Discussion. The marked decrease in serum prolactin levels in sham control animals was unexpected since previous reports have indicated that stress resulted in an increase in prolactin secretion (15, 16). A decrease in serum prolactin levels in sham and electrically stimulated rats, however, has also been observed by Kalra *et al.* (17) using different parameters for electrical stimulation. Our results confirm their observations. Clemens *et al.* (18), on the other hand, did not observe any change in serum prolactin levels for sham control animals and reported an increase in prolactin levels after electrical stimulation of the medial basal hypothalamus. The experimental protocol of these latter workers more closely followed that of Kalra *et al.* (17) than that of ours.

Although the blockage of α- and β-adrenergic fibers resulted in an increase in serum prolactin levels, the pattern of response was different. The more rapid action of propranolol may have been due to a more rapid distribution throughout the hypothalamus or due to an action on a different neuron cell population involved in prolactin regulation. The present experimental design does not allow for such a distinction to be made. Recent evidence indicates that adrenergic fibers are involved in inhibiting prolactin secretion since their blockage by a number of drugs results in a marked increase in serum prolactin (8, 9). Kamberi, Mical and Porter (10) has presented evidence that dopamine in small amounts (1.25 μg) was active in inhibiting prolactin secretion when infused into the third ventricle of the brain. Norepinephrine, on the other hand, did not inhibit serum prolactin levels unless high amounts were administered (100 μg). These same workers (19) also showed that the action of dopamine was on the CNS to release PIF rather than directly on the AP. Since dopamine has both α- and β-adrenergic activity in the periphery (20), the effect of blocking these neurons in the CNS may be due to blocking the action of dopamine on the release of PIF.

The influence of cholinergic fibers on prolactin secretion has not been extensively studied. Grosvenor and Turner (21) observed that atropine as well as Dibenamine blocked the AP prolactin release evoked by the nursing stimulus. Our laboratory has reported (22) that bilateral atropine implants into the

dorsal medial and ventral medial hypothalamus resulted in a significant increase in deciduomata formation which is indicative of an increase in prolactin secretion. The results of this report do not indicate that atropine increased prolactin release. A direct comparison, however, cannot be made between the two experiments because of the temporal difference (chronic vs acute), because the parameters of prolactin production were different (deciduomata formation vs RIA of serum prolactin) and because sites of injection were different (VMH-DMH vs third ventricle). Work is now in progress to elucidate the action of atropine on prolactin production.

Summary. The influence of neural blocking agents injected into the third ventricle of the brain of female rats at the time of vaginal estrus with and without electrical stimulation of the ventral medial hypothalamus (VMH) on serum prolactin levels was examined 30, 45, 75 and 135 min after initiation of electrical stimulation. Prolactin levels of sham control animals decreased 30–40% from initial values. Electrical stimulation of hypothalamus did not alter the response from that of sham controls. Intraventricular administration of atropine alone gave a response similar to that of sham controls while electrical stimulation modified the extent of the decrease. The injection of propranolol, a β-adrenergic blocker, with and without electrical stimulation, increased serum prolactin 100–110%. Phenoxybenzamine, an α-adrenergic blocker, alone resulted in a gradual but progressive increase in serum prolactin levels. Electrical stimulation of phenoxybenzamine-injected animals prevented the rise in serum prolactin observed for drug treatment alone.

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