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Effects of Intrahypothalamic Implants of Reserpine on Lactation and Pituitary Prolactin Content in the Rabbit.* (28546)

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Lactation is induced in the estrogen primed rabbit by systemic or intraventricular treatment with the tranquilizing agent, reserpine (1), apparently by antagonizing the inhibitory control exerted by the central nervous system over the release of pituitary prolactin (2). Similar effects on lactation are observed following the placement of small electrolytic lesions in the basal tuberal region of the hypothalamus(3) and such lesions have been found to deplete the hypophysis of prolactin (4). If a lesion has effectively activated lactation, subsequent treatment with reserpine neither activates the mammary glands nor depletes the hypophysis of prolactin(2). The results indicate that reserpine may exert its lactation inducing effect at this critical hypothalamic site. It is therefore of interest to apply reserpine directly to this site as well as directly to the hypophysis and study the effects of these treatments on the mammary glands and pituitary prolactin content.

Materials and methods. Twelve multiparous New Zealand white rabbits were ovariectomized on day 0 and treated with 0.1 mg estradiol benzoate subcutaneously daily for 10 days. On day 11 or 12 a minute amount of reserpine phosphate (<0.1 mg) was stereotaxically(5) implanted into the basal hypothalamus or hypophysis; 3 of the 12 rabbits were implanted with reserpine on day 0.

Reserpine implants were prepared as follows: the tip of a piece of 24-gauge tubing was carefully heated with reserpine phosphate powder and the melted reserpine was drawn into the tube. The outside of the tubing was carefully cleaned with ether, leaving only a small amount of solid reserpine visible on the tip. The recrystallized reserpine was hard and only very slowly soluble in physiological saline, but was effective in inducing a general depressant effect when homogenized, suspended in saline and injected subcutaneously. After stereotaxic implantation, the tube was fixed securely to the skull of the rabbit with dental cement and 2 small anchoring screws.

Autopsy was usually performed on day 18 or 19, from 6 to 8 days after reserpine implantation; but the 3 rabbits implanted on day 0 were autopsied 15 days later. The mammary glands were examined and graded by the Gardner-Turner classification(6) on a scale of 0 to 4 on days 0, 11 or 12 at implantation and autopsy on day 15, 18 or 19, and confirmed by histological study of biopsy and autopsy specimens. Histological methods of locating implantation sites in the brain and pituitary gland have been described earlier by Kanematsu and Sawyer(7). Prolactin content of each pituitary gland was assayed intradermally on 12 White King squabs approximately 4 weeks of age weighing 400-600 g(7). Standard samples of purified prolactin were assayed throughout the course of the investigation.

Results. In Fig. 1, the mammary glands of

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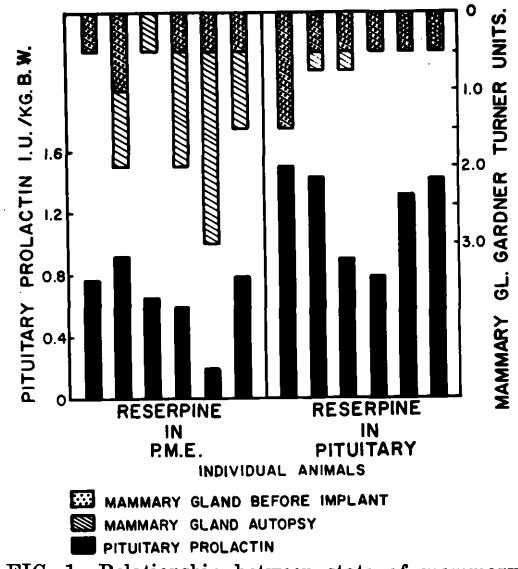

FIG. 1. Relationship between state of mammary gland and pituitary prolactin content of individual animals after implantation of reserpine into hypothalamus and hypophysis. Note that the 2 rabbits highest in mammary gland activation have pituitaries lowest in prolactin content.

5 of 6 animals were activated, 4 of them markedly, following implantation of reserpine into the posterior median eminence-posterior tuberal area (PME) of estrogen primed ovariectomized rabbits ($P<0.05$), while pituitary prolactin content was significantly low, 0.64 $\pm$ 0.10 IU/kg BW ($P<0.01$). Fresh milk was expressible from the teats of 4 of the 6 animals. In Fig. 1 the 2 PME cases with the highest mammary gland activation showed the lowest pituitary prolactin content. One animal failed to show any mammary gland activation in spite of a relatively low pituitary prolactin content, but her ovaries had been found to be extremely small, and presumably the mammary gland may not have been responsive. Little or no activation of the mammary glands was observed following implantation of reserpine into the hypophysis. The prolactin content of such pituitaries was high, 1.22 $\pm$ 0.12 IU/kg BW, but this is not significantly different from the 1.14 $\pm$ 0.25 IU/kg BW in estrogen primed ovariectomized control rabbits(4).

Little or no damage to brain tissue was observed to result from the direct implantation of reserpine in the PME area of the brain (Fig. 2). Furthermore, 5 of the tubes implanted in the hypophysis penetrated and remained in physical contact with PME without activating the mammary glands or depleting the pituitary prolactin content. These tubes may be considered as controls of the presence of stainless steel tubing in the PME implants. None of the rabbits showed any systemic effects of reserpine.

Fig. 3 shows the reconstructed localization of the reserpine implants. Five of 6 animals showed activation of the mammary glands, and 4 of these revealed depletion of pituitary prolactin when reserpine was placed in the PME area, while no change was observed following implantation into the adenohypophysis, neural stalk of the neurohypophysis, anterior

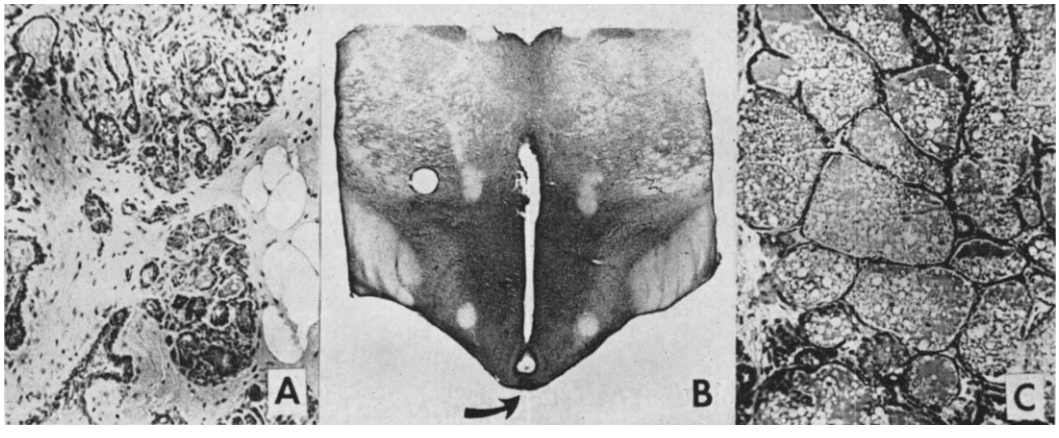

FIG. 2. Sections of mammary gland biopsies before (A) and 8 days after (C) implantation of reserpine into basal hypothalamus (B-near arrow). Actually, B is a frontal section of brain 0.4 mm anterior to implantation site, which corresponds to broken line in Fig. 3 (A, C, $\times$ 140; B, $\times$ 7).

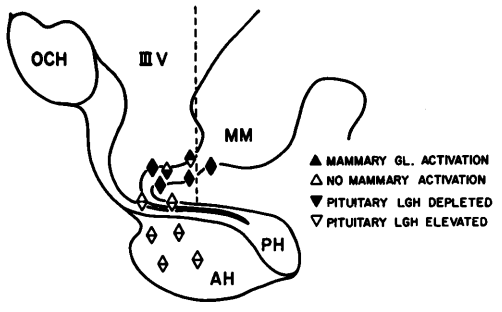

FIG. 3. Location of solid reserpine implants in hypothalamus and hypophysis, and effects on lactation and content of pituitary prolactin (LGH). Note that the high mammary gland activation (hypothalamic implant) is accompanied by low prolactin content.

median eminence or pituitary stalk. No significant difference was observed in the weights of hypophyses ($P<0.6$) or the left adrenals ($P<0.8$).

Discussion. Results of the present experiments confirm the well documented claim that reserpine activates lactation(8), and that it does so by causing release of prolactin from the adenohypophysis as proposed earlier by Kanematsu, Hilliard and Sawyer(2). The results also support the suggestion of Sawyer (1), based on intraventricular injection experiments, that the reserpine effect is exerted by its action within the brain, and, finally, that the site of this action is the posterior tuberal region of the hypothalamus as indicated by reserpine-lesion experiments(2). A local secretory influence of reserpine on pituitary gland cells is denied.

As might be expected from a local action of the drug, the implanted reserpine failed to produce systemic tranquilizing symptoms. The reserpine was still in a molecular state capable of exerting such pharmacological influences, for when the implant was dissolved and the solution injected systemically typical symptoms were observed. The local effect appeared to be one of slow absorption of the drug rather than physical destruction of the site by the implant: the development of lactational changes in mammary glands was slower than would have been caused by a lesion, and the implant was still effective when located up to half a millimeter behind the site required of an effective lesion. No deleterious influence on pituitary prolactin content was exerted by the penetration of, or mechanical contact with, PME by a stainless steel tube bearing a reserpine implant into the hypophysis or by the presence of the drug in the gland itself. In an earlier report(7), in which blank tubes were implanted into PME, they failed to affect mammary glands or pituitary prolactin content. In still other studies tips bearing adrenocortical steroids(9) or chronic electrodes (Kawakami and Sawyer, unpublished) in PME have also failed to influence the mammary glands. For these reasons the present results point to a local pharmacologic effect on hypothalamic neurons rather than the destruction of these cells.

Summary. Direct implantation of minute amounts of solid reserpine phosphate into the posterior median eminence-basal tuberal area of the hypothalamus initiated milk secretion and depleted the hypophysis of prolactin in the estrogen primed ovariectomized rabbit. Reserpine implantation into the hypophysis had no effect. The results indicate the focus of reserpine influence essential to the release of pituitary prolactin and activation of mammary glands is the posterior median eminence-posterior tuberal region of the hypothalamus.

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