EFFECT OF RESERPINE ON PITUITARY PROLACTIN CONTENT AND ITS HYPOTHALAMIC SITE OF ACTION IN THE RABBIT

By

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ABSTRACT

A single intravenous dose of reserpine (0.5 mg/kg) lowers the level of pituitary prolactin and induces lactation in ovariectomized, oestrogen-primed rabbits. These effects can also be produced by placing a discrete electrolytic lesion in the basal tuberal hypothalamus, and following such a lesion, reserpine exerts no further effects on pituitary prolactin or mammary gland activation. In contrast, when reserpine is administered to rabbits bearing lesions elsewhere in the hypothalamus, depletion of pituitary prolactin and lactation ensues. These results suggest that reserpine-induced prolactin depletion of the pituitary gland, with subsequent lactation, is mediated by a system within the basal tuberal hypothalamus.

Lactation in the rabbit following treatment with the tranquilizing agent, reserpine, was reported by Kehl et al. (1956) and independently by Sawyer (1957). This lactogenic effect of reserpine has been repeatedly confirmed in pseudopregnant and oestrogen-primed rabbits (Meites 1957; Tindal 1960; Khazan et al. 1962) as well as in rats (Benson 1958; Mayer et al. 1959). According to Meites (1958), pituitary prolactin levels are markedly increased following injection of a single large dose of reserpine. In the lactating rat, however, Moon & Turner (1959) reported that the pituitary prolactin content

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fell to 52% of the prenursing levels following reserpine treatment. The possibility that reserpine may stimulate the release of pituitary lactogenic hormone via a central nervous control mechanism was suggested by Sawyer (1957) following the observation that a minute amount of reserpine (0.25 mg), too small to evoke the effect when administered systemically, would induce mammary gland activation when injected into the third cerebral ventricle.

Recently, similar lactational effects have been achieved in oestrogen-primed rabbits by making small electrolytic lesions in the basal tuberal hypothalamus (Haun & Sawyer 1960, 1961). This is the same area in which electrical stimulation induces ovulation (Sawyer et al. 1963). In 1963, Kanematsu et al. reported that lesions which induce lactation cause a depletion in pituitary prolactin content.

The present experiments were designed to compare the mechanisms involved in reserpine-induced lactation with those of lesion-induced lactation. Consequently, hypothalamic lesions and reserpine treatment were employed separately and in combination. Comparisons were made on the basis of mammary gland activation and pituitary prolactin content. A preliminary report of this work has appeared earlier (Kanematsu et al. 1961).

**MATERIALS AND METHODS**

Twenty-nine New Zealand white rabbits (3.6–5.0 kg) were ovariectomized and divided into four groups (Table 1). Following ovariectomy, all rabbits received daily subcutaneous injections of 0.1 mg oestradiol benzoate in oil for 10 days.


*Group II*: Same treatment except that on day 12 each animal received 0.5 mg/kg reserpine, i. v.

*Group III*: Same treatment as Group II except that on day 1 a lesioning electrode was placed stereotaxically in the basal hypothalamus under pentobarbital anaesthesia.

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<th>Group</th>
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<td>IV</td>
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**Table 1.**

Experimental plan of treatment.
and an electrolytic lesion was made with direct current, 3 mA, 40 s (Sawyer et al. 1954).

**Group IV:** Lesions in this group were made on day 12. Mammary gland biopsies were taken on day 15. Starting on day 25, animals were reprimed with oestrogen for 10 days. On day 37 (12 days from start of repriming) they received reserpine. Autopsy day 40.

Mammary gland changes were assessed by the Gardner-Turner (1933) classification and by histological sections of biopsies taken before oestrogen priming (day 0), while placing lesions or administering reserpine (day 12) and on day 15. Mammary gland biopsies were fixed in Susa, embedded in paraffin, sectioned at 10 μ and stained with haematoxylin and eosin. Brains were fixed in formalin, embedded in gelatin, and frozen sections were stained with thionin before reconstruction. Location of the lesions was ascertained by observing the fresh brains under a dissecting microscope and by making graph paper reconstructions of brain sections.

Pituitary glands were weighed, quickly frozen on dry ice and stored in a deep freeze prior to prolactin assay. Prolactin content of each pituitary gland was assayed on 12 White King squabs approximately 4 weeks of age and weighing 400–600 g apiece. The intradermal assay method used has been described by Kanematsu & Sawyer (1963 a).

**RESULTS**

Results are summarized in Fig. 1. Control rabbits of Group I, which were injected with oestrogen alone, showed no significant activation of the mammary glands (0.66 ± 0.06 Gardner-Turner units at autopsy as compared with a starting mean of 0.56 ± 0.04 units). The terminal pituitary prolactin content of this group was 0.98 ± 0.21 IU/kg body weight.

The 7 rabbits of Group II, which had no brain lesions, were combined with 8 rabbits from Group III and IV which had lesions ineffective in activating mammary glands. Their lesion sites lay generally outside of the posterior basal tuberal-posterior median eminence area. Following treatment with reserpine the mammary glands of these animals were markedly activated: 1.53 ± 0.18 Gardner-Turner units from an initial 0.52 units to a terminal 2.05 units (Fig. 2 A). Their pituitary prolactin content was low: 0.51 ± 0.06 IU/kg body weight, only 52% as high as the control value (P < 0.02).

Four rabbits from Group III and two from Group IV showed marked activation of their mammary glands (1.21 ± 0.32 Gardner-Turner units) three days after the placement of electrolytic lesions in Group IV and 12 days in Group III. Their hypothalamic lesions invariably involved the posterior tuberal and premammillary areas (Fig. 3). Subsequent treatment with reserpine followed the schedule outlined above. As seen in Fig 1, reserpine did not significantly change the already-activated condition of the mammary glands (P < 0.2). The terminal pituitary prolactin content was, however, much higher than that of reserpine-treated non-lesioned rabbits (P < 0.001) and not significantly different from control values (P < 0.3).
Pituitary prolactin content and mammary gland activation in response to reserpine in hypothalamic-lesioned and non-lesioned rabbits. »Mammary gland activation« is the net change in condition of the glands from their pre-treatment status. Numbers at the base of the bars indicate the number of animals used.

* Significantly different from controls, $P < 0.02$.
** Significantly different from reserpine treated non-lesioned group, $P < 0.001$.
** Not significantly different from controls, $P < 0.3$.
+ vs ++ Not significantly different, $P < 0.2$. 

Fig. 1.
DISCUSSION

Earlier studies of the effects of hypothalamic lesions on activation of the mammary glands in rabbits (Haun & Sawyer 1960, 1961) were initiated in search of an explanation of the mechanism by which reserpine stimulates lactation. The results of those studies and others (McCann & Friedman 1960; Grosz & Rothballer 1961) were consistent with the hypothesis of Desclin (1950) and Everett (1956) who proposed that the secretion of prolactin from the
pituitary gland is held chronically in check by the hypothalamus. Prolactin assays of the pituitary glands revealed that effective hypothalamic lesions lower the pituitary prolactin content and suggested that the intact hypothalamus chronically inhibits release rather than synthesis of prolactin (Kanematsu et al. 1963). Moreover, the present reserpine-lesion-prolactin assay data suggest that reserpine may act like a functional lesion in antagonizing the hypothalamic mechanism which chronically inhibits release of pituitary prolactin: if the critical site has already been destroyed by an electrolytic lesion reserpine can exert no further influence on the release of prolactin. Reserpine, however, may still exert its stimulatory effect on ACTH secretion (Brodie et al. 1961) and possibly on other hormones influencing lactation. Hypothalamic lesions at sites other than the basal tuberal region neither activate lactation themselves nor block the effectiveness of reserpine from doing so.

The effect on pituitary prolactin exerted in the oestrogen-primed rabbit by acute treatment with reserpine is release of the hormone from the hypophysis. In this respect the present results confirm the findings of Moon & Turner (1959), who reported that in the rat reserpine treatment of the lactating animal decreased pituitary prolactin content by 48% of the prenursing level. Neither of these studies supports the earlier report of Meites (1958), that reserpine greatly increases the pituitary prolactin content in the oestrogen-primed rabbit.

The long-term influence of an optimally placed lesion appears to be quite different from the acute release effect. A new equilibrium for prolactin synthesis and release must be instituted under these conditions, for it is possible, after prolonged priming with oestrogen, to have both an activated mammary gland and a pituitary high in prolactin content. The pituitary gland released from hypothalamic control by the lesion may be stimulated directly by oestrogen to produce and release prolactin just as pituitary cells in tissue culture have recently been shown to do when oestrogen is added to the medium (Nicoll & Meites 1962). Similar long-term effects may well be induced by chronic treatment with reserpine.

The critical site of action of reserpine in its influence on lactation appears to be the basal tuberal hypothalamic area. The earlier results of Sawyer (1957) in which reserpine injected into the third cerebral ventricle activated lactation are consistent with this idea. Quite recently Kanematsu & Sawyer (1963 b) have found that minute amounts of solid reserpine (0.1 mg), when implanted into the posterior tuberal area, depleted the hypophysis of prolactin and activated the mammary glands without noticeable destruction of brain tissue.

The critical site to which reference was made above is also the area controlling release of ovulating hormone in the rabbit (Sawyer et al. 1963). In this connection it is of interest that systemic treatment with reserpine did not appear to block release of pituitary ovulating hormone in response to direct electrical stimulation of this site (Saul & Sawyer 1957). However, reserpine has
been shown to exert inhibitory influences on gonadotrophic function from rodents (Barraclough & Sawyer 1957) to primates (Erikson et al. 1960) and a hypothalamic site of action seems not unlikely. In the rat the same dose of reserpine which blocks 100% of the animals from ovulating if administered at prooestrous (Barraclough & Sawyer 1957) activates a maximal release of prolactin-luteotrophin if administered on the first day of dioestrous (Barraclough & Sawyer 1959). These and other endocrine effects of reserpine have been reviewed by Gaunt et al. (1961).

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REFERENCES


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