The effect of reserpine on pituitary-adrenocortical function in the rat

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Although the effect of reserpine on hypothalamo-pituitary-adrenocortical (HPA) function has been extensively studied there is still a great deal of confusion in the literature about this aspect of its action. Pituitary adrenocortical activity was assessed in male Sprague-Dawley rats and changes in adrenal ascorbic acid (Roe & Kuether, 1943) and plasma corticosterone (Zenker & Bernstein, 1958) concentrations were used as indices of corticotrophin (ACTH) release. One hour after a single intraperitoneal injection of either 1.25 or 2.5 mg/kg of reserpine adrenal ascorbic acid was depleted and the plasma corticosterone concentration was elevated. The effect persisted for 24 h suggesting that the drug causes prolonged hypersecretion of ACTH. Increased pituitary adrenocorticotrophic activity was evident in rats treated with the same volume of vehicle (1.0% glacial acetic acid in deionized water, pH 4) 1 h but not 24 h later. After 5-7 repeated, single, daily injections of the same doses of reserpine it was found that adrenal ascorbic acid and plasma corticosterone concentrations were normal 1 h after the final injection, indicating that the alkaloid no longer acted as a stressful stimulus, i.e. some form of 'adaptation' occurred. No similar 'adaptation' was observed in response to injection of vehicle alone. Rats 'adapted' to reserpine were exposed to cold (4° C for 1 h) either immediately or 24 h after the final injection. In animals 'adapted' to the higher dose of reserpine ACTH release no longer occurred and no change was seen in the concentrations of adrenal ascorbic acid and plasma corticosterone when the stress was applied immediately after the final injection. However, there was also an apparent inhibition of ACTH release in the corresponding vehicle treated rats. Twenty-four hours after the final injection the application of cold stress caused a rise in the plasma corticosterone concentration *without* a concomitant depletion of adrenal ascorbic acid in reserpine treated rats. The stress response in the corresponding vehicle treated controls was normal. It appears that, in certain conditions, reserpine can effectively inhibit the functional activity of the HPA system but in practice the use of different parameters of ACTH secretion and the neglect of proper controls can lead to widely different interpretations of the experimental data.

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Oestrogen dependence of enzyme activity and intracellular structure in the hamster submaxillary gland

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Recently we analysed the androgen dependence of individual estero-proteases and cell structures in the mouse submaxillary gland (Bhoola, Dorey & Jones, 1973). Changes in the activity of chymotrypsin- and trypsin-like enzymes and renin correlated closely with variations in the granule population of the secretory tubules of the mouse gland. In contrast, the estero-protease kallikrein and secretory organelles in the acinar cells showed no such dependence on androgens. Consequently, experiments were designed to determine whether kallikrein activity and the granule population in acinar cells of the mouse and hamster are influenced by oestrogens. No such relationship has been demonstrated for ovarian hormones previously, even though the hamster shows a sialomucin sex-hormone linked dimorphism (Shackleford & Klapper, 1961). In the present communication we report our findings in the hamster.

Estero-protease activity in the hamster submaxillary gland can be ascribed mainly to kallikrein; it was measured on benzoyl L-arginine ethyl ester (BAEe) and expressed as specific activity ($\Delta_{E_{366}}$/min)/mg protein, $n = 8$. The kallikrein activity (BAEe) increased post-natally from 2.91 in the male and 0.83 in the female (18-22 days old) to 9.88 in the male and 14.83 in the female (88-92 days old). Ovariectomy resulted in a fall in

BAEe activity which was restored after replacement of oestrogen (0.5 mg oestradiol undecylate x 4 doses/7 day interval) and unaffected by androgen (5 mg testosterone oenanthate x 4 doses/7 day interval).

Morphologically mature glandular units in rodents consist of acini, intercalated ducts, secretory tubules and striated tubules. Postnatal development of glandular elements other than the acinus shows no sex-linked variation in the hamster. Ultrastructurally, sex hormone related differences are only observed in the granule population of the acinar cells. In the female, mainly one type of cell is seen with a single population of granules which possess a central pale area and a peripheral dense region. In contrast, the male has three cell types, the predominant containing granules with a central dense core and an outer pale section. In the ovariectomized females the fine structural appearance of the granules changes to that observed in the male, but the *female* type of granules return after replacement of oestrogen.

Androgens specifically influence enzyme induction and formation of granules in the secretory tubules of the mouse submaxillary gland. In the hamster in contrast the sex-hormone related changes in the acinar cell granules and in the activity of the kallikrein-like enzymes seems to be mediated by oestrogens. Such changes are probably regulated by genes specifically controlled by androgen or oestrogen.

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Effect of oestrogens and progesterone alone and in combination on the female rat plasma kininogen concentrations

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Previous work in this laboratory (McCormick & Senior, 1973) has confirmed the observations of Weigerhausen, Kläusch, Hennighausen & Sosat (1967) that the concentration of plasma kininogen rises with advancing gestation in the rat. McCormick & Senior (1971) have also shown that oestrogens in optimal doses raise the concentration of plasma kininogen in the female rat, and progesterone in the doses studied had no effect.

This communication describes experiments to study the effect of oestrogens and progesterone alone and in combination on non-pregnant female rats in an attempt to elucidate this phenomenon further.

All drugs were administered daily for five days to mature, virgin, female rats by the subcutaneous route. The concentrations of plasma kininogen were determined using the micromethod of Diniz & Carvahlo (1963).

β-oestradiol (in doses from 1 to 50 μg/kg/day) and a soluble synthetic oestrogen, FI6173 (2,4,dimethyl-5,5-diphenyl pent-4-enoic acid sodium salt) (in doses from 10-500 μg/kg/day) were used.

With both oestrogens a dose response effect was obtained with maximum significant increase in plasma kininogen concentrations occurring at 5 μg/kg for β-oestradiol and 50 μg/kg for FI6173. Progesterone had no effect on kinin precursor concentrations at doses of 0.5, 2.5 and 5 mg/kg; however, a significant decrease was obtained with 10 mg/kg. β-oestradiol and progesterone were administered in arachus oil.

Two dose levels of oestrogens were used (5 μg/kg and 10 μg/kg for β-oestradiol and 50 μg/kg and 250 μg/kg for FI6173) in conjunction with varying doses of progesterone (0.5, 2.5, 5 and 10 mg/kg). In all cases, the significant increase in kininogen concentrations produced by the oestrogens was reduced by, and in proportion to, the dose of progesterone. Progesterone had a more marked effect when in conjunction with the lower dose of each oestrogen.

The results show that the two oestrogens used increase the plasma kininogen concentration of the non-pregnant female rat in proportion to the dose used. A high dose of progesterone is required to produce a significant decrease in plasma kininogen concentrations.

It appears that progesterone will reduce the increase in kinin precursor produced by the