be required for optimal ova implantation in rodents[23], a concept in accord with the findings of this study, in which we showed a greater than 50% reduction in pregnancies in female mice chronically treated with CB-154 during mating. Furthermore, mean number of pups per litter in the ergot-treated group was not significantly different from that in the controls, suggesting that the antifertility activity of CB-154 is not exerted by a direct effect on either the blastocyst or embryo, but by selective inhibition of ova implantation. Pregnancy was not, however, totally prevented in the ergot-treated mice but was significantly delayed. Nearly all of the mice in this group that eventually became pregnant, did so in the last 10 days of the 50-day treatment period. A slight adaptation to chronic treatment with the drug is, therefore, suggested by these results. The luteotrophic action of prolactin is the most probable mechanism by which this hormone influences ova implantation in rodents[22–24].

CB-154 has been previously shown to effectively inhibit lactation in rats[25], rabbits, pigs, dogs[4] and man[8], but has been shown to be essentially ineffective in suppressing established lactation in cows[6]. We have provided evidence in this study that the ergot is also effective in inhibiting lactation in mice. Pup mortality and rate of growth during lactation were markedly increased and decreased, respectively, in the CB-154 treated groups. The lactating dams treated with the ergot, as well as the other ergot-treated mice, showed no apparent ill effects of the treatment such as changes in body weight, activity, etc., when compared to controls. On the other hand, surviving weanling mice derived from the ergot-treated lactating dams were weak and stunted, indicating severely suppressed lactation.

The notable anti-mammary tumor effects of CB-154 in rodents[3, 14–16] and a potential role indicated for prolactin in human breast tumorigenesis[17–19], have prompted a number of clinicians to contemplate and/or actively use this drug for treatment of metastatic carcinoma of the breast[26, 27]. The drug has also been used, and appears to be potentially very effective, in suppressing non-puerperal galactorrhea in women[8]. The results of the present study provide evidence that chronic treatment with the drug in mice does not, upon drug withdrawal, induce sustained irreversible inhibition of fecundity and lactation. Whether or not use of the drug in primates will induce adverse irreversible effects on these processes remains to be determined. It is most unlikely that CB-154 would alter fecundity in primates as prolactin is not luteotrophic in these species[24].

Zusammenfassung. C3H/HeJ-weibliche Mäuse, die vor der Paarung dauernd mit CB-154, einem stark wirkenden Mittel zur Unterdrückung der Prolactinsekretion, behandelt wurden, wiesen keine bedeutsame Schädigung ihrer Fortpflanzungsfähigkeit auf. CB-154 Dauerbehandlung von weiblichen Mäusen sowohl während der Paarung als auch während der Laktation führte zu einer bedeutsamen Herabsetzung der Fruchtbarkeit und Milchabsonderung.

C. W. Welsch[28] and Linda K. Morford[29, 30]

Department of Anatomy, Michigan State University, East Lansing (Michigan 48824, USA), 15 July 1974.

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Effect of Sulpiride on Prolactin Release by Rat Pituitaries in vitro

It has been recently demonstrated that N-(ethyl-1-pyrrolidinil-2) methylmethoxy-2-sulfamoyl-5-benzamide (sulpiride), a tranquilizing drug, can induce galactorrhea in humans[1]. It has also been demonstrated that sulpiride induced a decrease of serum gonadotropin levels in postmenopausal women[2]. In the rat, sulpiride modified serum gonadotropin and prolactin levels[3]. The aim of the present investigation was to demonstrate whether sulpiride can act directly on the pituitary gland. This possibility was investigated by incubating pituitary glands in vitro in the presence of sulpiride.

Materials and methods. Adult male rats of the Wistar strain were used as pituitary donors. They were killed by decapitation, the pituitary gland exposed, and the neural lobe was discarded. The pituitary gland was then cut into 2 approximately equal pieces, each being placed in a different beaker containing 5 ml of medium TC 199 (Difco). Each beaker contained 5 pituitary halves. The glands were preincubated for 1 h at 37 °C, being gassed continuously with a mixture of oxygen (95%) and carbon dioxide (5%) in a Dubnoff metabolic shaker. The medium was then discarded, being replaced by new medium. A small volume (0.2 ml) containing either ot the following solutions was added to the beakers: a) saline, b) 1 mg of sulpiride sulphate, c) 0.05 mg of sulpiride sulphate, d) a rat hypothalamic extract equivalent to 1.5 hypothalami, e) a rat hypothalamic extract equivalent to 1.5 hypothalami plus 1 mg of sulpiride sulphate, and f) a rat hypothalamic extract equivalent to 1.5 hypothalami plus 0.05 mg of sulpiride sulphate. 3 beakers for each treatment were used. The incubations were carried out for 4 additional h. At the end of the incubations, the media were separated frozen, and kept at −20 °C until assayed. The wet weight of the 5 pituitary halves contained in each beaker was recorded. Prolactin was assayed in the medium using the double-antibody radioimmunoassay described by Niswender et al.[4], with materials distributed by the National Institute of Arthritis and Metabolic and Digestive Diseases, Bethesda, USA. The results were expressed as ng of NIAMDD-Rat Prolactin-RP 1 per mg of wet pituitary weight and per ml of medium. The

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Effect of sulpiride and hypothalamic extracts on prolactin release by rat pituitaries in vitro

Group and treatment [a]	Dose	Prolactin concentration [b]
1 Saline	—	217.6 ± 25.2 [c]
2 Sulpiride	1 mg	272.2 ± 31.0
3 Sulpiride	0.05 mg	352.0 ± 59.4 [d]
4 Rat hypothalamic extract	1.5 hypothalami	120.0 ± 4.4
5 Rat hypothalamic extract plus sulpiride	1.5 hypothalami + 1 mg	221.0 ± 41.2
6 Rat hypothalamic extract plus sulpiride	1.5 hypothalami + 0.05 mg	209.6 ± 36.0

[a] Three beakers containing 5 pituitary halves per each treatment were used. [b] Expressed as ng of NIAMDD - rat prolactin-RP, 1 mg of pituitary weight/ml of medium. [c] The variance analysis showed statistical significance of the differences among groups ($p < 0.05$). [d] Group 3 vs Group 1: $p < 0.05$ (Duncan's new multiple range test).

significance of the differences among groups was tested by means of the analysis of variance and the Duncan's new multiple range test [5].

Results. The analysis of variance showed that there is a significant difference in the concentration of prolactin in the media from the 6 different treatments investigated ($p < 0.05$). The Table shows that the media to which sulpiride was added, contained significantly higher concentrations than the control media where saline was added. The addition of rat hypothalamic extracts inhibited the release of prolactin. On the other hand, the media where hypothalamic extracts plus sulpiride were added contained higher concentrations of prolactin than those where only hypothalamic extracts were added. Due to the rather high dispersion of these values however, the Duncan test did not show the presence of statistical significance among them.

Discussion. It is a well known fact that the anterior pituitary gland is under hypothalamic control[6]. In the case of prolactin, this influence is mainly inhibitory[7]. When the anterior pituitary gland is removed from its normal location in the pituitary fossa and incubated in vitro, it releases increasing amounts of prolactin[8]. In our experiment, it seems evident that in such a circumstance sulpiride was able further to stimulate the prolactin release. Paradoxically, the lower dose used seemed to be more effective than the higher one in stimulating prolactin release. On the other hand, it is evident that the addition of sulpiride simultaneously with rat hypothalamic extracts inhibited to some extent the supressive effect of the prolactin release-inhibiting factor (PIF) contained in them on prolactin release. It thus seems evident that the action of sulpiride is exerted, at least in part, at the pituitary level. The results of this investigation do not exclude, however, the possibility that some of the effects of sulpiride reported in vivo could have been exerted also at the hypothalamic level[9].

Résumé. La sulpiride stimule la libération de la prolactine de l'hypophise après 4 h d'incubation. L'addition d'extraits hypothalamiques l'inhibe, mais la sulpiride ajoutée en même temps aux extraits hypothalamiques permet l'inversion de cet effet d'inhibition. Il semble evident que la sulpiride peut modifier la libération de la prolactine en agissant directement sur l'hypophise du rat, in vitro.

L. DEBELJUK, H. DASKAL, R. ROZADOS and A. GUITELMAN

Centro de Investigaciones sobre Reproducción, Facultad de Medicina, Universidad de Buenos Aires, Paraguay 2155, Buenos Aires (Argentina); Instituto Nacional de Tecnología Agropecuaria (I.N.T.A.); and Departamento de Endocrinología, Hospital T. Alvarez, Buenos Aires (Argentina), 3 May 1974.

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Stimulation and Suppression of Somatomedin Activity by Serotonin and Melatonin

The pineal gland hormone, melatonin, and its indoleamine precursor, serotonin, were recently shown to exert opposing effects on the secretion of growth hormone in both the rat[1,2] and in man[3]. These effects of melatonin and serotonin were proposed as one way by which the pineal might regulate growth[2]. The present study was undertaken to determine whether the actions of these two indoles might extend to regulation of growth hormone action as well as its release from the pituitary gland.

Growth hormone is believed to exert its effects on skeletal growth via an intermediary substance which was originally called 'sulphation factor'[4]. More recently the general term 'somatomedin' was proposed[5] to include those substances having growth promoting activity

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