LH seen in intact mature female rats[21]. It has been suggested that the anovulatory effect of hyperprolactinemia may be due to a reduction or blockade of this positive feedback action[22]. However, efforts to demonstrate or confirm this mechanism with experimental models of hyperprolactinemia have produced contradictory or inconclusive results. Studies in intact rats have shown that hyperprolactinemia can reduce, but not prevent, the positive feedback effect of estradiol[4,18]. It is not clear whether this reduction contributed to the acyclicity in these animals, however, due to the pseudopregnancy state. This complication can be avoided when ovariectomized animals are utilized. The only other known study employing chronic ovariectomized rats also failed to find any effect of elevated plasma prolactin levels on estrogen-induced release of LH[11], but the hyperprolactinemia was of rather short duration (10 days). The present study, which compared the effects of short term and long-term hyperprolactinemia, thus provides more definitive information on whether inhibition of steroid-induced release of LH can result from prolonged elevation of plasma prolactin levels. Taken together with the studies cited above, the present investigation strongly suggests that chronic hyperprolactinemia does not prevent estrogen-induced positive feedback in the female rat and thus this animal model is probably not adequate for studying mechanisms which underlie this effect observed in hyperprolactinemic patients.

1 To whom requests for reprints should be sent. Present address: Department of Pharmacology and Toxicology, University of Louisville, Louisville, KY 40292, USA.

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Peptide-stimulated release of prolactin from the fowl anterior pituitary gland

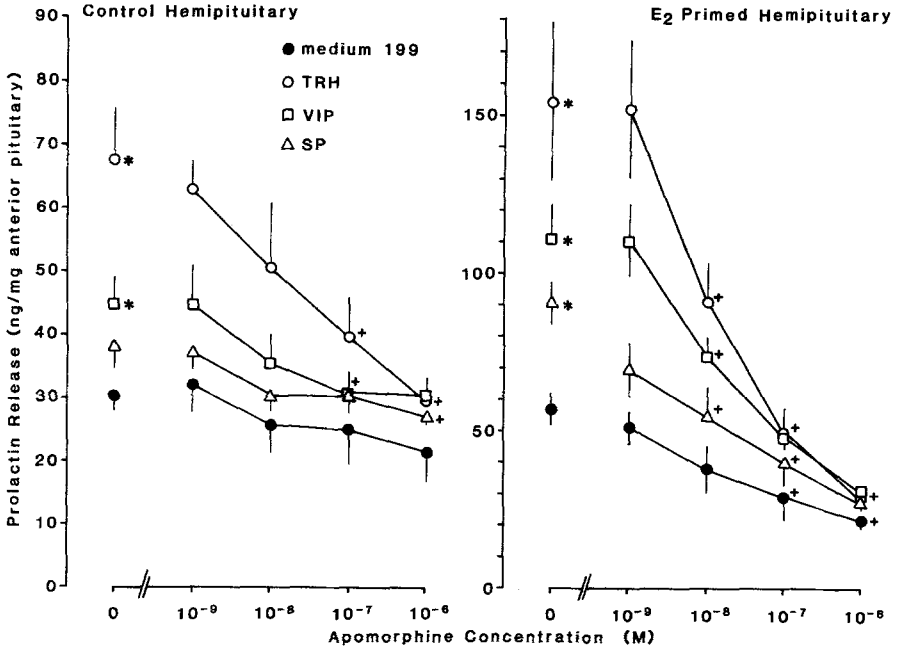
T.R. Hall* and A. Chadwick

Department of Pure and Applied Zoology, The University of Leeds, Leeds LS29JT (England), 28 March 1984

Summary. Anterior pituitary glands from broiler fowl were preincubated for 24 h in either medium 199 only or medium containing estradiol 17β, following which they were incubated in medium containing thyrotrophin releasing hormone (TRH), vasoactive intestinal polypeptide (VIP) or substance P (SP), alone or with the dopamine agonist, apomorphine. Estradiol priming stimulated release of prolactin and enhanced apomorphine-inhibition of prolactin release. TRH stimulated prolactin release, an effect reversed by apomorphine, and priming with estradiol potentiated both effects. VIP stimulated prolactin to a lesser degree and again this was inhibited by apomorphine and potentiated by estradiol. SP had little effect on the nonsteroid-primed pituitary, but stimulated release of prolactin after estradiol treatment, though less effectively than TRH or VIP.
Key words. Prolactin release, stimulation of; vasoactive intestinal polypeptide; substance P; thyrotrophin releasing hormone; estradiol 17β, effect on fowl pituitary responsiveness.

A number of peptides have been isolated from the mammalian hypothalamus that are able to affect the release of anterior pituitary gland hormones[1,2]. It is believed that these neuropeptides are released from nerve terminals into the hypophyseal portal system, where they are transported to the anterior pituitary cells[3,4]. Some of these peptides, including thyrotrophin releasing hormone (TRH), vasoactive intestinal polypeptide (VIP) and substance P (SP) stimulate the release of prolactin in vitro[2,5–7]. In birds, the effects of TRH on anterior pituitary function are well-documented[8–11], but little attention has been paid to the actions of other neuroactive peptides[12,13]. Recently, large quantities of VIP and SP have been detected, by radioimmunoassay and immunofluorescence techniques, in the central nervous system and particularly the hypothalamus and median eminence of domestic fowl. In addition, VIP stimulated the release of immunoassayable prolactin when administered to domestic fowl[14–16]. However, little is known concerning the interactions of prolactin stimulating and inhibiting agents in birds. The effects of the peptides (TRH, VIP and SP) on release of prolactin from fowl pituitary glands under different conditions in vitro were determined in the present study.

Materials and methods. Procedure. Anterior pituitary glands were dissected from the heads of freshly-killed 8- to 10-week-old broiler fowl and collected in ice-cold medium 199. The pituitaries were bisected and each half placed in a small plastic tube with 100 µl medium 199 or medium containing 10^{-8} M estradiol 17β. After through gassing with 95% O_2/5% CO_2 the tubes were sealed and placed in a water bath at 39 °C for 24 h. The medium was then aspirated and discarded. Fresh medium, containing the test substances as described in the results section, was then added. After gassing and sealing the tubes were incubated for a further 3 h, following which the medium was removed, diluted 10-fold and stored at −20 °C until assayed. Assay and data analysis. The homologous chicken prolactin



Effects of apomorphine on peptide-stimulated release of prolactin from fowl pituitary glands in vitro. Hemi-adenohypophyses were preincubated for 24 h in medium alone (left-hand panel) or 10^{-8} M estradiol 17β (right-hand panel), following which they were incubated for 3 h in medium alone (●) or in medium containing 10^{-7} M TRH (○) 10^{-7} M VIP (□) or 10^{-7} M SP (△) alone or with various concentrations of apomorphine. Results show mean ± SEM (n = 5). *p < 0.05; compared with incubation in medium only. +p < 0.05; compared with incubation in medium without apomorphine (analysis of variance).

radioimmunoassay of Scanes et al.[17] and Lea et al.[18] was employed. This assay has an intraassay variability of 2.5% and a lower detection limit of 2.4 ng/ml. All samples were assayed together to avoid interassay variability. Results are expressed as mean ± SE of five replicate observations. Differences between hemipituitary pairs were analyzed by Student's t-test. Differences between various experimental treatments were compared by analysis of variance and Duncan's multiple range test, with a level of significance of $p < 0.05$.

Results and discussion. Control and estrogen-primed hemipituitaries were incubated with different concentrations of apomorphine, either by itself or together with 10^{-7} M TRH , VIP or SP. Prolactin concentrations in the medium are shown in the figure. As shown in the left-hand panel, with control hemipituitaries release of prolactin was not significantly affected by apomorphine alone, though there was a trend towards inhibition of release with increasing concentration of the drug. Both VIP and TRH significantly stimulated ($p < 0.05$) prolactin release from control hemipituitaries, and in incubations containing these peptides apomorphine inhibited prolactin release in a concentration-related fashion. Release of prolactin was slightly, but non-significantly, elevated in the presence of SP in control incubations. The right-hand panel shows prolactin release from estrogen-primed hemipituitaries. Even without peptides in the medium, prolactin release was markedly elevated ($p < 0.01$) compared to the control hemipituitary, and was inhibited by apomorphine in a concentration-dependent manner. All three peptides significantly stimulated release of prolactin, with TRH the most effective ($p < 0.01$) and SP the least effective ($p < 0.05$), and apomorphine inhibited release of prolactin by an amount related to its concentration. At 10^{-6} M apomorphine, prolactin concentrations were similar to those seen in control, non-steroid-primed incubations, and were significantly lower ($p < 0.05$) than from estrogen-primed pituitaries incubated in medium only.

A number of observations can be made on these results. It appears that all three peptides are able to stimulate the release of prolactin directly from the pituitary gland of the domestic fowl under certain conditions. While the prolactin-stimulating action of TRH is well-known[9-13], we believe that this is the first time that VIP and SP have been shown to stimulate prolactin release from the pituitary gland of an avian species. Estrogen priming apparently not only stimulates release of prolactin, as it does in mammals[19], but also enhances the responsiveness of the pituitary gland to both stimulatory (peptides) and inhibitory (apomorphine) agents.

The role of dopamine in the control of prolactin secretion in birds is obscure, as both inhibitory effects[11-13,20] and complete lack of effects[21] have been reported in the past. Recently this question has been reexamined and convincing evidence for a dopaminergic prolactin-inhibiting mechanism has been presented both in vivo[22] and in vitro[23]. As we have reported earlier[11,12] and as is shown here, dopaminergic inhibition of prolactin may be significant only during times of enhanced secretion of the hormone.

These results suggest that estrogens may play an important role in determining the magnitude of hormonal responses to hypothalamic agents. We have previously shown that estradiol 17β can stimulate prolactin release in vitro[24,25] but these results show, in addition, an increased responsiveness of the pituitary gland. Whether this is due to the increased secretion of prolactin only, or whether steroid-pretreatment induces an increase in receptors to the peptides and apomorphine, is not known at present.

In conclusion, TRH, VIP and SP, which are present in high concentrations in the hypothalamus of the domestic fowl, can all stimulate release of prolactin from the fowl pituitary gland in vitro, particularly following exposure of the pituitary gland to estrogen. This allows the possibility of a role for these peptides in the control of prolactin secretion.

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* Present address: Wolfson Institute, The University of Hull, Hull HU6 7RX.

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Effect of aminoglutethimide on murine fetal hepatic erythroid colony formation[1]

P. Leung[2] and A.S. Gidari

Department of Pharmacology, Box 29, State University of New York, Downstate Medical Center, 450 Clarkson Avenue, Brooklyn (New York 11203, USA), 21 May 1984

Summary. Pretreatment of pregnant mice with aminoglutethimide phosphate, an inhibitor of glucocorticoid synthesis, increases the content of fetal liver erythroid colony-forming cells (CFU-E), as assessed by the formation of erythroid colonies in vitro by fetal liver cells in plasma clots containing exogenous erythropoietin. In addition, the inability of aminoglutethimide to influence erythroid colony formation in vitro suggests that endogenous glucocorticoids exert a suppressive effect on the number of functional CFU-E in the fetal liver.
Key words. Mice, pregnant; aminoglutethimide phosphate; mice, liver, fetal; erythroid colony-forming cells.

During fetal development, the liver is a major site of erythropoiesis in mammals[29,35]. Several studies have demonstrated that erythropoiesis in the fetal liver is regulated by erythropoietin[31,40]. Other hormones (e.g. testosterone) may also modulate erythropoiesis in utero by enhancing the production of erythropoietin[39] or increasing the sensitivity of fetal liver cells to erythropoietin[9]. In contrast, glucocorticoids appear to suppress fetal liver erythropoiesis in vivo[5,23,26,27]. In addition, fetal livers from dexamethasone-pretreated pregnant rats contained a reduced number of morphologically identifiable erythroid progenitor cells[36]. Similarly, pretreating pregnant mice with dexamethasone reduced the number of functional erythroid colony-forming cells (CFU-E)[20], the immediate progenitor of identifiable erythroblasts[16,19]. In these studies, the CFU-E can be functionally identified by its ability to form small colonies of erythroid cells in a semi-solid medium containing low concentrations of exogenous erythropoietin after 2 days of culture[25,34]. The ability of dexamethasone to inhibit erythroid colony formation in vivo suggests that inhibitors of adrenal steroid biosynthesis might reverse or abrogate the inhibitory effect of glucocorticoids on the fetal liver CFU-E. In this connection, aminoglutethimide, a compound which inhibits the formation of 20α-hydroxycholesterol from cholesterol[10,14], and therefore reduces endogenous glucocorticoid levels, was employed in an attempt to identify a potential role for endogenous glucocorticoids in the modulation of fetal liver erythropoiesis. Thus pregnant mice were injected i.v. with 1 mg of aminoglutethimide phosphate or 0.9% NaCl and, 24 h later, the formation of erythroid colonies by fetal liver erythroid progenitor cells in plasma clots containing erythropoietin (25 mU) was assessed.

Materials and methods. Chemicals. Aminoglutethimide and water-soluble aminoglutethimide phosphate were obtained from Ciba Pharmaceuticals (Summit, NJ).

Human urinary erythropoietin, processed by Dr Peter P. Dukes at the Hematology Research Laboratory, Children's Hospital of Los Angeles, was provided by the Division of Blood Diseases and Resources of the National Heart, Lung and Blood Institute, National Institutes of Health. The specific activity of this material was estimated to be 110.9 IU/mg protein.

Animals. CD-1[Crl:CD-1(ICR)BR] mice (Charles River Breeding Laboratories, Inc., Wilmington, MA), maintained on Rat Chow 5012 (Ralston Purina Co., St. Louis, MO) and water ad libitum, were mated and examined daily for vaginal plugs. The morning on which a vaginal plug was found was designated as the first day of gestation. To assess the effect of treatment with aminoglutethimide on erythroid colony formation by fetal liver cells in vitro, pregnant mice were injected i.v. with either 0.2 ml vehicle (0.9% NaCl) or 0.2 ml vehicle containing 1 mg of aminoglutethimide phosphate 24 h prior to collection of fetal livers. The mice, at 15 days of gestation, were killed by cervical dislocation on the morning of each study in order to minimize the potential effects of diurnal variations in circulating glucocorticoid levels. The individual fetuses were excised and placed in RPMI-1640 (Microbiological Associates, Inc., Walkerville, MD).

Erythroid colony assay. Erythroid colonies were grown in plasma clot cultures, and quantitated essentially as described in McLeod et al.[25] and previously reported by Leung and Gidari[21].

Statistical analysis. Statistical analysis of the data was performed by employing a one-way analysis of variance[33] to ascertain the presence of any significant difference among the group means. Subsequently, if significant differences were present, the Newman-Keuls test[33] was used to establish which pairs of means were significantly different. In some experiments, when