NEUROTRANSMITTER EFFECTS ON RELEASE OF PROLACTIN AND GROWTH HORMONE IN VITRO FROM PITUITARY GLANDS OF THE PIGEON, COLUMBA LIVIA

T. R. HALL*
Department of Biology, Marquette University, Milwaukee, Wisconsin 53233, U.S.A.

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SUMMARY

Single pigeon anterior pituitary glands were incubated with or without a hypothalamus in media containing various drugs. Release of prolactin and growth hormone was quantified by an electrophoretic-densitometric method. The hypothalamus stimulated release of both prolactin and growth hormone from the pituitary gland. Dopamine did not affect hormone release from pituitary glands incubated alone, but inhibited hypothalamus-stimulated release of prolactin and augmented hypothalamus-stimulated release of growth hormone in a dose-related manner. The effects of dopamine were reversed by its antagonist, pimozide. Serotonin stimulated release of prolactin and inhibited release of growth hormone from pituitary–hypothalamus co-incubations, and these effects were blocked by its antagonist, methysergide. Thyrotrophin releasing hormone (TRH) stimulated release of both hormones directly from pituitary glands incubated alone. Dopamine now inhibited TRH-stimulated release of prolactin, without affecting TRH-stimulated release of growth hormone. These results indicate that the neurotransmitters, dopamine and serotonin, affect the in-vitro release of factors from the hypothalamus which control the secretion of prolactin and growth hormone. In addition, dopamine may inhibit release of prolactin directly from the pituitary gland, but only when secretion of prolactin is high initially.

INTRODUCTION

The prolactin-stimulating activity of the avian hypothalamus has been well documented. Since Kragt & Meites (1965) and Nicoll (1965) first detected prolactin-releasing activity in the hypothalamus of the pigeon and tricoloured blackbird, similar findings have been reported for the turkey, blackbird, duck, quail and domestic fowl (Chen, Bixler, Weber & Meites, 1968; Nicoll, Fiorindo, McKenney & Parsons, 1970; Tixier-Vidal & Goudjii, 1972; Hall, Chadwick, Bolton & Scanes, 1975; Knight & Chadwick, 1975; Hall & Chadwick, 1982). The avian hypothalamus may contain prolactin-inhibiting activity also (Nicoll et al. 1970; Tixier-Vidal & Goudji, 1972; Knight & Chadwick, 1975; Bolton, Chadwick, Hall & Scanes, 1976) and a growth hormone-releasing principle (Müller, Sawano & Schally, 1968; Hall & Chadwick, 1975).

Some mammalian releasing and release-inhibiting hormones have been synthesized and affect the release of avian pituitary hormones. Thyrotrophin releasing hormone (TRH) stimulates release of avian thyroid-stimulating hormone, prolactin and growth hormone in vitro (Scanes, 1974; Hall et al. 1975) and in vivo (Harvey, Scanes, Chadwick & Bolton, 1978). Synthetic mammalian somatostatin inhibits release of growth hormone from fowl pituitary glands incubated in vitro (Hall & Chadwick, 1975, 1976).

* Present address: Department of Pure and Applied Zoology, University of Leeds, Leeds LS2 9JT.
In mammals, release of the hypothalamic hormones may be regulated by brain neurotransmitters (Clemens, 1976; MacLeod, 1976). The effects of neurotransmitters, in particular the monoamines, on release of pituitary hormones in birds has received some study. Tixier-Vidal & Gourdi (1972) suggested that reserpine, a monoamine storage depletor, stimulates release of prolactin, indicating the presence of a prolactin-inhibiting factor as in mammals. Hall & Chadwick (1982) found that l-dihydroxyphenylalanine inhibited release of prolactin from fowl pituitary–hypothalamus co-incubations. Nistico, Germana, Ciriaco, Bronzetti, Rotiroti & Scapagnini (1979) and Nistico, Germana, Ciriaco & Bronzetti (1980) have shown that centrally acting drugs affect secretion of prolactin in vivo in the pigeon, as judged from ultrastructural observation of crop sac development. The effects of the monoamines, dopamine and serotonin, on release of prolactin and growth hormone from pigeon pituitary glands in vitro have now been studied.

MATERIALS AND METHODS

Adult pigeons of both sexes were obtained from commercial sources. They were maintained under an 18 h light : 6 h darkness photoperiod at 22 °C with free access to food and water for 2 weeks before use. They were killed by cervical dislocation when the pituitary glands and hypothalami were removed within 2 min and incubated according to the method of Hall et al. (1975) and Hall & Chadwick (1982). Each anterior pituitary gland or anterior pituitary gland plus a whole hypothalamus was preincubated for 1 h at 39 °C in 100 µl Medium 199 (Gibco, Grand Island, New York, U.S.A.). This medium was discarded and fresh medium containing the experimental treatments was added. The incubation was carried out for 5 h in 95% O2/5% CO2. Three experiments were performed: in the first, dopamine hydrochloride (Regis Chemical Co., Morton Grove, Illinois, U.S.A.; 1, 10 or 100 ng/ml) alone or with pimozide (McNeil, Fort Washington, Pennsylvania, U.S.A.; 100 ng/ml) was added to pituitary and pituitary–hypothalamus incubations; in the second, serotonin creatinine sulphate (Regis Chemical Co.; 1, 10 or 100 ng/ml) alone or with methysergide maleate (Sandoz Pharmaceutical Division, Hanover, New Jersey, U.S.A.; 1 µg/ml) was added to pituitary and pituitary–hypothalamus incubations; in the third, dopamine hydrochloride (100 ng/ml), TRH (Beckman, Palo Alto, California, U.S.A.; 100 ng/ml) or both compounds were added to pigeon pituitary incubations. The monoamines were dissolved in 1% ascorbic acid and the other drugs were dissolved in distilled water, with the exception of pimozide, which was dissolved in 0.1 M-tartaric acid. Control incubations contained equivalent amounts of ascorbic acid and tartaric acid. The medium was stored at -20 °C until assayed. Prolactin and growth hormone in the samples of medium were separated by polyacrylamide gel electrophoresis (Hall et al. 1975). The prolactin (Nicoll & Nichols, 1971; Scanes, Chadwick & Bolton, 1976) and growth hormone (Nicoll & Licht, 1971; Harvey & Scanes, 1977) bands were quantified by densitometry after amido-black staining of the proteins.

RESULTS

Pigeon anterior pituitary glands were incubated in medium containing various concentrations of dopamine or dopamine plus pimozide, either alone or with a hypothalamus. Addition of a hypothalamus to an anterior pituitary incubation resulted in a significant increase in release of prolactin (Table 1). Dopamine did not significantly affect release of prolactin when added to a pituitary incubation, but inhibited the hypothalamus-stimulated release of prolactin in a dose-related manner. Pimozide, while not affecting prolactin release by itself, reversed the actions of dopamine on release of prolactin by pituitary glands incubated with a hypothalamus. The presence of the hypothalamus also caused a significant increase in release of growth hormone from the pituitary gland, an effect
augmented by dopamine in a dose-related manner (Table 1). Dopamine, at the doses used, did not affect release of growth hormone from pituitary glands incubated without hypothalami. The stimulatory action of dopamine on hypothalamus-mediated release of growth hormone was inhibited by pimozide.

Table 1. Effects of dopamine and pimozide (100 ng/ml) on release of prolactin and growth hormone from pigeon anterior pituitary glands (AP) co-incubated with hypothalami (Hypo). Results are expressed in arbitrary density units/mg wet weight pituitary gland ± S.E.M. (n = 6)

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Prolactin (density units/mg)‡</th>
<th>GH (density units/mg)‡</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>AP only</td>
<td>AP + Hypo</td>
</tr>
<tr>
<td>Medium only</td>
<td>25 ± 2</td>
<td>38 ± 3*</td>
</tr>
<tr>
<td>Dopamine (1 ng/ml)</td>
<td>27 ± 2</td>
<td>39 ± 4*</td>
</tr>
<tr>
<td>Dopamine (10 ng/ml)</td>
<td>23 ± 4</td>
<td>30 ± 4</td>
</tr>
<tr>
<td>Dopamine (100 ng/ml)</td>
<td>25 ± 3</td>
<td>26 ± 3†</td>
</tr>
<tr>
<td>Pimozide</td>
<td>26 ± 4</td>
<td>36 ± 3*</td>
</tr>
<tr>
<td>Pimozide + dopamine (1 ng/ml)</td>
<td>22 ± 6</td>
<td>40 ± 4*</td>
</tr>
<tr>
<td>Pimozide + dopamine (10 ng/ml)</td>
<td>24 ± 4</td>
<td>39 ± 3*</td>
</tr>
<tr>
<td>Pimozide + dopamine (100 ng/ml)</td>
<td>26 ± 5</td>
<td>36 ± 4</td>
</tr>
</tbody>
</table>

⁎P < 0.05 compared with incubation of the pituitary gland alone; †P < 0.05 compared with incubation of the pituitary gland plus hypothalamus in medium only (Student’s t-test).
‡Hormones were measured by densitometry following polyacrylamide gel electrophoresis.

Table 2. Effects of serotonin and methysergide (1 μg/ml) on release of prolactin and growth hormone from pigeon anterior pituitary glands (AP) co-incubated with hypothalami (Hypo). Results are expressed in arbitrary density units/mg wet weight pituitary gland ± S.E.M. (n = 6)

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Prolactin (density units/mg)‡</th>
<th>GH (density units/mg)‡</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>AP only</td>
<td>AP + Hypo</td>
</tr>
<tr>
<td>Medium only</td>
<td>29 ± 2</td>
<td>44 ± 3*</td>
</tr>
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<td>Serotonin (1 ng/ml)</td>
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<tr>
<td>Serotonin (100 ng/ml)</td>
<td>28 ± 3</td>
<td>62 ± 6†</td>
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<tr>
<td>Methysergide</td>
<td>26 ± 3</td>
<td>45 ± 5*</td>
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<tr>
<td>Methysergide + serotonin (1 ng/ml)</td>
<td>30 ± 2</td>
<td>50 ± 6*</td>
</tr>
<tr>
<td>Methysergide + serotonin (10 ng/ml)</td>
<td>25 ± 2</td>
<td>54 ± 2*</td>
</tr>
<tr>
<td>Methysergide + serotonin (100 ng/ml)</td>
<td>28 ± 3</td>
<td>56 ± 6*</td>
</tr>
</tbody>
</table>

⁎P < 0.05 compared with incubation of the pituitary gland alone; †P < 0.05 compared with incubation of the pituitary gland plus hypothalamus in medium only (Student’s t-test).
‡Hormones were measured by densitometry following polyacrylamide gel electrophoresis.

In a second incubation, anterior pituitary glands and hypothalami were incubated with various doses of serotonin, alone or with methysergide. Release of prolactin and growth hormone after these treatments is summarized in Table 2. Neither serotonin nor methysergide affected hormone release directly from the pituitary gland. Serotonin stimulated release of prolactin in pituitary–hypothalamus co-incubations. A dose-related effect was noted, though a significant increase in prolactin content of medium samples occurred only with the highest dose of serotonin. Serotonin inhibited hypothalamus-stimulated release of growth hormone. Methysergide did not affect hypothalamus-
stimulated release of prolactin and growth hormone by itself, but blocked the actions of serotonin on release of prolactin and growth hormone.

In a third incubation, pigeon anterior pituitary glands were incubated with dopamine, TRH, or both compounds, and release of prolactin and growth hormone was measured. Dopamine by itself did not affect hormone release from the pituitary gland (control incubation, prolactin 24 ± 6 (S.E.M.) density units/mg, growth hormone 81 ± 10 density units/mg v. dopamine-treated (100 ng/ml), prolactin 20 ± 5 density units/mg, growth hormone 90 ± 7 density units/mg, n = 6). Thyrotrophin releasing hormone significantly (P < 0.05) stimulated release of both prolactin (50 ± 8 density units/mg, n = 6) and growth hormone (120 ± 10 density units/mg, n = 6). Addition of dopamine reduced (P < 0.01) the release of prolactin compared with TRH alone (30 ± 6 density units/mg, n = 6) whereas dopamine had no apparent effect on TRH-stimulated growth hormone release (115 ± 8 density units/mg, n = 6).

**Discussion**

From mammalian studies it is believed that the mechanism triggering the release of the hypothalamic hormones involves the brain neurotransmitters. Changes in neurotransmitter output and in particular alterations in secretion of the monoamines, dopamine, noradrenaline and serotonin, have been associated with many hormonal changes in mammals (Chen, Mueller & Meites, 1974; Advis, Simpkins, Chen & Meites, 1978). There is a large body of evidence indicating that dopamine is involved in the control of prolactin secretion in mammals. Drugs which decrease secretion of dopamine have been found to increase secretion of prolactin, and conversely, drugs which increase secretion of dopamine reduce the secretion of prolactin (MacLeod, 1976). *In vitro*, dopamine inhibited the release of prolactin directly from the mammalian pituitary gland, an effect reversed by the receptor blocker, pimozide (Clemens, 1976). Dopamine has been reported to possess both growth hormone releasing and inhibiting activities in mammals, although these effects are mediated through the hypothalamus (Martin, 1976). Serotonin is known to stimulate prolactin release in mammals, through a hypothalamic mechanism (Clemens, 1976) and similarly, serotonin appears to stimulate release of mammalian growth hormone (Martin, 1976). The serotonin receptor blocker, methysergide, inhibited these responses.

Reserpine, which is known to induce prolactin release in mammals, presumably by depleting the prolactin-inhibiting monoamine, dopamine (MacLeod, 1976), also appears to stimulate avian prolactin secretion (Tixier-Vidal & Gourdjii, 1972). Nistico et al. (1979, 1980) administered a variety of neuroleptic drugs to pigeons and measured the crop sac response as an index of circulating prolactin levels. They found that drugs that inhibited the dopaminergic system stimulated secretion of prolactin. The present results confirm that monoamines are capable of affecting pituitary release of prolactin and growth hormone in a pituitary–hypothalamus co-incubation system and suggest a possible role for the endogenous putative neurotransmitters, dopamine and serotonin, in the hypothalamus. Since neither monoamine altered hormone release directly from the unstimulated pituitary gland, this would suggest that they act through a hypothalamic mechanism, presumably on releasing and/or release-inhibiting hormones. However, dopamine was capable of inhibiting TRH-stimulated release of prolactin, but not of growth hormone, directly from the pituitary gland. This effect is similar to that reported previously (Bolton et al. 1976; Hall & Chadwick, 1982) where rat hypothalamic extract was found to inhibit release of prolactin from chicken pituitary glands, but only if they were stimulated to release prolactin with chicken hypothalamic extracts. In contrast to the above results Border & Chadwick (1977) reported that serotonin stimulated release of prolactin directly from chicken pituitary glands *in vitro*. Whether this is due to species differences or assay differences is unclear. They used an homologous fowl prolactin radioimmunoassay to measure release of hormone, a technique much more sensitive than densitometry.
These results show that prolactin secretion in the pigeon may be under inhibitory dopaminergic control, though dopamine may play a role only when secretion of prolactin is high. Serotonin appears to stimulate prolactin secretion through an undefined hypothalamic mechanism. This may be a common feature of vertebrates, as similar results have been found in mammals (Clemens, 1976), amphibians (G. Uruña & T. R. Hall, unpublished observations) and teleosts (Oliveau, 1978; Olcse, Hall, Figueroa & de Vlaming, 1979). Control of growth hormone secretion in the pigeon appears to differ from that in mammals, since in the pigeon dopamine stimulates, whereas serotonin inhibits, release of pituitary growth hormone through a hypothalamic mechanism. Whether the neurotransmitters act through a growth hormone-releasing (Hall & Chadwick, 1975) or a growth hormone-inhibiting (Hall & Chadwick, 1975, 1976) factor is not known at present.

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REFERENCES


