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MUSCARINIC INHIBITION OF PROLACTIN PRODUCTION IN CULTURES OF RAT PITUITARY CELLS

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SUMMARY

Acetylcholine inhibits prolactin production from cell cultures of rat pituitary glands with a half-maximal effect at about 0.2 μM, and from GH-cells, clonal strains of rat pituitary cells, with a half-maximal effect at about 1 μM. The inhibition ranges between 80 and 40 % of control values. Inhibition is detectable at 2 hours, and continues for days in the presence of the anticholinesterase, eserine. Muscarinic agonists mimic the cholinergic inhibition and nicotinic agonists do not. The inhibition is blocked by atropine, a muscarinic antagonist, and not by hexamethonium, a nicotinic antagonist.

INTRODUCTION

Binding sites for muscarinic agonists of acetylcholine have been identified in the anterior pituitary gland (1). Acetylcholine, 25 μM, or 10 μM carbachol inhibits prolactin release from cell cultures of pituitary glands (2); these reports present conflicting data about the ability of atropine to block the inhibition. We show here that prolactin production is inhibited by acetylcholine not only in cells cultured from pituitary glands, but also in GH-cells, cell lines from a rat pituitary tumor which produce prolactin (3). This inhibition has muscarinic characteristics.

MATERIALS AND METHODS

Cell culture: Male Sprague Dawley rats, 175-200 g, were used to prepared dispersed cell cultures from the anterior pituitary glands. Dispersion of the cells was the same as

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Table I: Effect of eserine and acetylcholine on prolactin production from GH-cells

	Prolactin μg hormone/ml medium
Control	22 ± 2
Acetylcholine, 50 μM	21 ± 1
Eserine, 50 μM	21 ± 1
Acetylcholine, 50 μM + eserine, 50 μM	13 ± 1

GH-cells were incubated for 2 days in the presence of the indicated compounds and then the medium collected and assayed for prolactin. Each value is the average of duplicates ± the range.

previously described (4), except after the pieces had been rinsed with soybean trypsin inhibitor, we incubated the pieces in 0.01 mg/ml Viokase, 4 NF, for 1 to 1.5 hours before dispersing the cells. Experiments were begun 3 days after plating the cells.

We plated 2×10^5 CH_4C_1 cells in each 35 mm culture dish and changed the medium every few days. Medium was changed on the day before the experiment was begun, and cells were usually used 9 days after plating.

Both GH-cells and the cells from pituitary glands were kept in a humidified atmosphere of 95% air, 5% CO_2 in 1.5 ml per dish of Ham's F10 medium containing 2.5% fetal calf serum and 15% horse serum. At the start of each experiment, medium was changed and concentrated solutions of compounds to be tested were added in 0.05 ml volumes. Unless otherwise indicated, 50 μM eserine was present in the medium in experiments that used acetylcholine. Most of the results given in this paper have been reproduced in experiments similar to the ones shown here.

Prolactin assay: Prolactin was assayed by microcomplement fixation as previously described (3). Dr. A.F. Parlow provided rat prolactin (RP-1) through the hormone distribution program of NIAMDD.

Materials: Acetylcholine bromide or iodide, dimethylphenylpiperazinium, and hexamethonium were from Sigma, St. Louis, Missouri. Oxotremorine and muscarine were from Regis, Morton Grove, Illinois. Eserine sulfate was from ICN, Cleveland, Ohio. Atropine was from K + K Labs, Inc., Hollywood, California, and carbachol from Aldrich, Milwaukee, Wisconsin.

RESULTS

Data in Table I show that the presence of eserine, an anticholinesterase agent, was necessary for acetylcholine to inhibit prolactin production in GH-cells, presumably because eserine prevents the breakdown of the neurotransmitter. Eserine had no effect on prolactin production. In all subsequent experiments using acetylcholine, 50 μM eserine was also added to the medium.

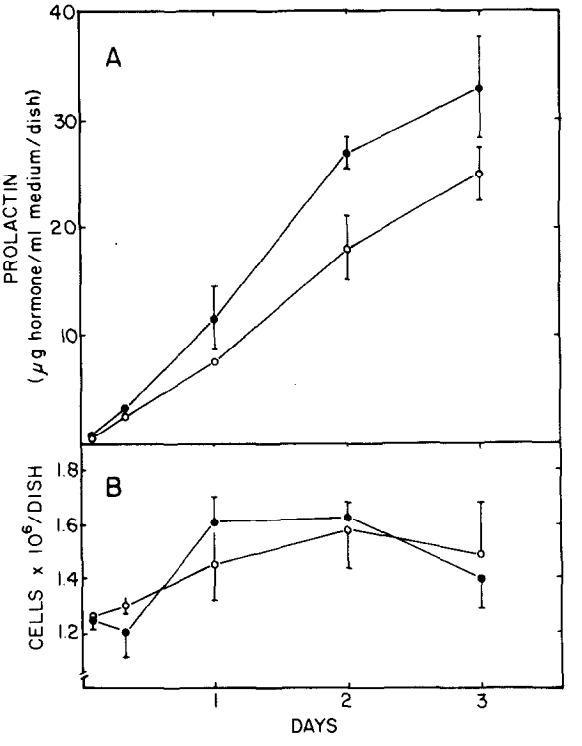

Fig. 1. Time course of the response of GH-cells to acetylcholine. Medium and cells were collected at the indicated time intervals. 1A. Accumulation of prolactin in the medium. 1B. Cell number per dish. Each point represents the mean of triplicates and the bars indicate the SD, except where the symbol was larger than the deviation. ●--● control, o--o 10 μM acetylcholine.

In this experiment prolactin production was inhibited to 60% of control values. The amount of inhibition in other experiments with GH-cells ranged from 50 to 80% of controls.

The inhibition of prolactin production by GH-cells lasted for days in the presence of acetylcholine (Fig. 1A). The amount of hormone accumulated in the medium was reduced compared to control values, but not completely prevented. Prolactin production was inhibited to 74% of control values at 2 hours and this amount of inhibition remained relatively constant over the next 3 days. The inhibition was not a toxic effect since the number of cells in culture was not affected (Fig 1B). After 72 hours of

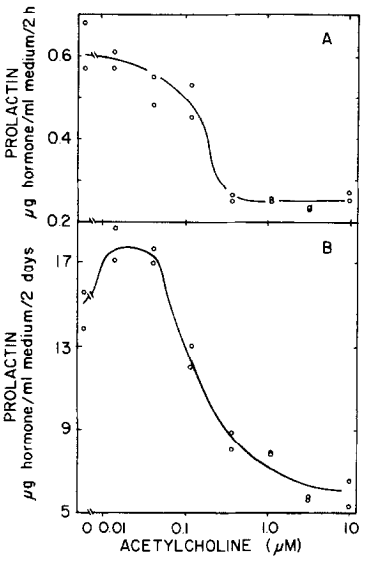

Fig. 2. Effect of acetylcholine on prolactin production by cell cultures from anterior pituitary glands. 2A. Medium which was collected after a 2 hour incubation. 2B. Medium was replaced on the cells after the 2 hour incubation, and collected after a 2 day incubation. The slight increase in prolactin production at low acetylcholine concentrations was not seen reproducibly.

treatment, treated cells contained 2.0 ± 0.3 μg prolactin/10^6 cells, and control cells contained 2.7 ± 0.2 μg prolactin/10^6 cells (mean ± SD). Therefore acetylcholine did more than just inhibit release. Acetylcholine must either decrease the synthesis of prolactin, cause intracellular prolactin to be degraded, or use a combination of these processes to prevent prolactin which does not appear in the medium from accumulating in the cells.

The inhibition of prolactin production from cells from pituitary glands after 2 hours or 2 days of treatment is shown in Fig. 2. Prolactin production was maximally inhibited to 50% of control values in this experiment; in other experiments with cells from pituitary glands, maximal inhibition ranged from 40 to 70% of controls. Half-maximal inhibition occurred at about 0.2

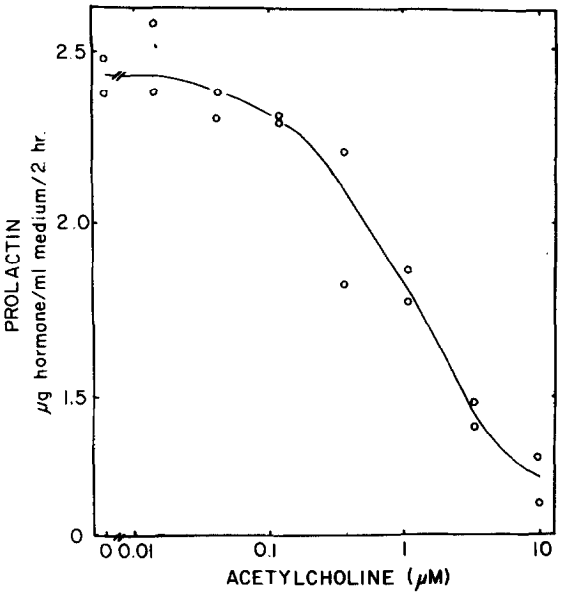

Fig. 3. Effect of acetylcholine on prolactin production by GH-cells. The medium was collected after a 2 hour incubation period. Similar results were seen after a 2 day incubation period.

μM and was the same at 2 hours or 2 days. Similar concentrations inhibited prolactin production by the GH-cells (Fig. 3), although these cells appeared somewhat less sensitive since half-maximal inhibition occurred at about 1 μM.

The inhibition had the characteristics of a process mediated by muscarinic receptors (5). Data in Table II show the inhibition caused by 10 μM acetylcholine was completely prevented by 0.1 uM atropine, a muscarinic antagonist. Atropine also reversed inhibition by acetylcholine in normal cells. Hexamethonium, which blocks nicotinic transmission at autonomic ganglionic cells, did not block the acetylcholine-induced inhibition. Data in Table III show that muscarine and the muscarinic agonist oxotremorine inhibited prolactin production. Carbachol, which has both muscarinic and nicotinic actions, also inhibited hormone production. In other experiments we found the

Table II: Effect of antagonists on inhibtion of prolactin production

	Prolactin μg hormone / ml medium
Experiment 1	
Control	71 ± 3
Acetylcholine, 10 μM	27 ± 2
Acetylcholine, 10 μM + 1 μM atropine	73 ± 3
Acetylcholine, 10 μM + 0.1 μM atropine	74 ± 7
Acetylcholine, 10 μM + 0.01 μM atropine	60 ± 1
Experiment 2	
Control	80 ± 2
Acetylcholine, 10 μM	39 ± 3
Acetylcholine, 10 μM + 1 μM atropine	81 ± 10
Hexamethonium, 100 μM	86 ± 10
Acetylcholine, 10 μM + 100 μM hexamethonium	42 ± 3

GH-cells were incubated for 2 days in the presence of the indicated compounds, and the accumulation of prolactin in the medium over that time period is shown. Each value is the average of duplicates ± the range.

same concentrations of nicotine and dimethylphenylpiperazinium, which are nicotinic agonists, did not cause inhibition.

Discussion

We have shown that cells from pituitary glands are sensitive to acetylcholine, responding to concentrations of less than 1 μM. In addition, we have shown that GH-cells, clonal strains of rat pituitary cells, also respond to acetylcholine. Bang and Gautvik (6) reported that acetylcholine did not affect prolactin

Table III: Effect of cholinergic agonists on inhibition of prolactin production

	Prolactin μg hormone/ ml medium
Experiment 1	
Control	71 ± 3
Acetylcholine, 30 μM	45 ± 2
Muscarine, 100 μM	52 ± 8
Muscarine, 10 μM	54 ± 1
Oxotremorine, 100 μM	44 ± 3
Oxotremorine, 10 μM	61 ± 8
Carbachol, 100 μM	54 ± 5
Carbachol, 10 μM	47 ± 11

GH-cells were incubated for 2 days in the presence of the indicated compounds, and then the medium collected and assayed for prolactin. Each value is the average of duplicates ± the range.

production by GH-cells during a 20 hour period. The lack of effect in their experiments and not ours probably occurred because they did not add an anticholinesterase. It is unlikely acetylcholine has any role in normal regulation of prolactin, although the direct effect of acetylcholine on the pituitary cells may explain at least part of the effects of cholinergic agonists, which can inhibit release of prolactin in intact animals (7,8).

The mechanism by which acetylcholine inhibits prolactin production is not known. Acetylcholine has been shown to stimulate phosphatidyl inositol labelling in the anterior pituitary gland (9), a process which appears to be associated with increased calcium permeability. Acetylcholine causes growth hormone release from the pituitary gland (9); the phosphatidyl inositol effect may be the mechanism by which this hormone is released. It is unlikely calcium entry into the cell caused by acetylcholine is the mechanism that inhibits prolactin release, since causing calcium entry into the cell stimulates prolactin release (10). It seems more likely prolactin release is inhibited because acetylcholine inhibits adenylate cyclase, as it has been shown to in other systems (11). The lack of effect of acetylcholine on cyclic AMP levels in the pituitary gland (9) may be because the gland contains several cell types. The GH-cells will provide a less heterogeneous system to study the mechanism of muscarinic inhibition.

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