Brazilian Journal of Medical and Biological Research (1999) 32: 1561-1564
ISSN 0100-879X

Chronic effect of antidopaminergic drugs or estrogen on male Wistar rat lactotrophs and somatotrophs

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M.C. Oliveira is the recipient of a CNPq fellowship.

Received December 2, 1998
Accepted September 29, 1999

Abstract

The aim of the present study was to evaluate the effect of antidopaminergic agents on the somatotrophs in the presence of hyperprolactinemia. Adult male Wistar rats were divided into 6 groups: a control group and five groups chronically treated (60 days) with haloperidol, fluphenazine, sulpiride, metoclopramide or estrogen. Somatotrophs and lactotrophs were identified by immunohistochemistry and the data are reported as percent of total anterior pituitary cells counted. The drugs significantly increased the percentage of lactotrophs: control (mean ± SD) 21.3 ± 4.4, haloperidol 27.8 ± 2.2, fluphenazine 34.5 ± 3.6, sulpiride 32.7 ± 3.5, metoclopramide 33.4 ± 5.5 and estrogen 42.4 ± 2.8. A significant reduction in somatotrophs was observed in animals treated with haloperidol (23.1 ± 3.0), fluphenazine (22.1 ± 1.1) and metoclopramide (24.2 ± 3.0) compared to control (27.3 ± 3.8), whereas no difference was observed in the groups treated with sulpiride (25.0 ± 2.2) and estrogen (27.1 ± 2.8). In the groups in which a reduction occurred, this may have simply been due to dilution, secondary to lactotroph hyperplasia. In view of the duplication of the percentage of prolactin-secreting cells, when estrogen was applied, the absence of a reduction in the percent of somatotrophs suggests a replication effect on this cell population. These data provide additional information about the direct or indirect effect of drugs which, in addition to interfering with the dopaminergic system, may act on other pituitary cells as well as on the lactotrophs.

Key words
- Somatotrophs
- Lactotrophs
- Estrogen
- Antidopaminergic drugs

Introduction

In rats, prolactin-producing cells vary in number under different physiological and pathological stimuli. Under the action of drugs, the replication of these cells occurs in parallel to changes in hormone secretion, i.e., both increase in the presence of estrogen or dopaminergic antagonists (1) and are reduced in the presence of dopaminergic agonists or progestagens (2). The effect of these drugs on the somatotroph population is less clear. Whereas Lloyd (3) reported a relative decrease in the number of somatotrophs with the use of estrogen, Ho et al. (4) quantitatively demonstrated a hyperplastic effect of estrogen on the somatotrophs as well as on other pituitary cells. Objective information about the cellularity of somatotrophs during the use of antidopaminergic agents is not

available. Thus, the objective of the present study was to determine the percent of somatotrophs in rats with hyperprolactinemia induced by estrogen or other antidopaminergic agents such as haloperidol, fluphenazine, sulpiride and metoclopramide.

Material and Methods

The study was conducted on 47 adult male Wistar rats maintained under conditions of light from 7:00 a.m. to 7:00 p.m., temperature-controlled environment and free access to water and rat chow throughout the experiment. The animals were divided into 6 groups, i.e., a control group (N = 9) and five groups submitted to the following treatments: haloperidol (N = 7), fluphenazine enantate (N = 8), sulpiride (N = 7), metoclopramide (N = 9) or estradiol valerianate (N = 7). Metoclopramide and haloperidol were obtained through the State Health Department (Secretaria da Saúde do Estado), fluphenazine through the National Health Department (Central de Medicamentos do Ministério da Saúde), and sulpiride and estradiol were obtained from commercial suppliers. Haloperidol and sulpiride were diluted in 0.9% NaCl and estradiol in corn oil. Metoclopramide and fluphenazine were used undiluted. Haloperidol, sulpiride and metoclopramide were administered intraperitoneally on a daily basis at doses of 2, 50 and 20 mg/kg, respectively. The esterified formulations were administered subcutaneously once a week at doses of 35 mg/kg (fluphenazine) and 300 µg/rat (approximately 1200 µg kg^{1} $week^{-1}$; estradiol). The animals that did not receive haloperidol, sulpiride or metoclopramide were injected daily with 0.9% NaCl, 1 ml/kg intraperitoneally; the rats that did not receive fluphenazine or estradiol were injected weekly with corn oil, 1 ml/kg, subcutaneously. The duration of treatment was 60 days. At the end of this period the rats were decapitated without anesthesia and the total pituitary gland was rapidly removed, weighed and fixed in 10% formalin.

The pituitaries were then embedded in paraffin and the blocks were serially cut into 4- to 6-µm thick sections. The entire tissue available was cut, resulting in approximately 100 sections in 80% of cases. Three slides of each pituitary gland, from cuts 25, 50 and 75 of each rat, were treated with PAS orange G. The best section was selected, and the slides immediately preceding and following it were used for the immunohistochemical technique using the avidin-biotin-peroxidase method (5). Primary anti-growth hormone (GH) serum (anti-rGH-IC 1) was obtained from the National Institute of Diabetes and Digestive and Kidney Disease (NIDDK), University of Maryland School of Medicine, and used at 1:400 dilution in phosphate-buffered saline (PBS). The anti-prolactin (PRL) primary antiserum, produced in rabbits immunized with ovine prolactin, was obtained from Escola Paulista de Medicina and used at 1:500 dilution.

Target cells (somatotrophs or lactotrophs) were counted under the light microscope at 40X magnification, using a grid lens. The observer was unaware of the treatment group to which the animal belonged. At least 200 cells per pituitary were examined and cells containing the identified hormone were counted. The results were expressed as percentage of cells containing GH (or cells containing PRL) in relation to total cellularity.

Data were analyzed statistically by one-way ANOVA followed by the multiple amplitude Duncan test. The Spearman r coefficient was used to determine the correlations. The level of significance was considered to be $P<0.05$ in all tests performed.

This study was approved by the Ethics Commitee of the institution.

Results

The mean values of the somatotroph percentages and the respective confidence intervals are listed in Table 1. The same data

for lactotrophs are listed in Table 2. A significant reduction in somatotroph percentage was observed in the groups treated with haloperidol, fluphenazine and metoclopramide when compared to the control group. A significant increase in lactotroph percentage was observed in all treated groups compared to control and this increase was greater in the estrogen group than in the groups treated with the other antidopaminergic drugs.

The correlation coefficient between somatotroph and lactotroph percentages was 0.09 for haloperidol, -0.37 for fluphenazine, 0.58 for sulpiride, 0.01 for metoclopramide and 0.50 for estrogen. The critical value of the coefficient was not significant in all cases.

Discussion

The number of somatotrophs are relative and should be interpreted in the light of the transformations undergone by lactotrophs. An increase in lactotroph percentage was observed with all the antidopaminergic agents tested, in agreement with the known hyperprolactinemic effect of these drugs (6). Thus, the reduction in the percentage of somatotrophs observed with haloperidol, fluphenazine or metoclopramide was within the expected limits regarding prolactin-secreting cell hyperplasia. This decrease was possibly of a dilution type, although some interesting features should be pointed out. The first one is that the increase of lactotrophs in these groups ranged from 30 to 62%, whereas the reduction of somatotrophs was 11.4 to 19.1%. As these two cell populations are preponderant in the anterior pituitary, it is unlikely that the repercussions of drug treatments on the corticotrophs, gonadotrophs and thyrothrophs account for the total difference. Second, if we consider that the decrease is only relative, we should expect an inverse correlation between the percentage of somatotrophs and the percentage of lactotrophs. This inverse correlation was only observed in the fluphenazine group, but even then the critical value of the correlation coefficient was not significant. Only the evaluation of cells producing all pituitary hormones, or the counts of absolute cell number could explain this fact, ruling out the possibility of a direct action of the drugs on the somatotrophs. Also with respect to the antidopaminergic agents, there was no significant reduction in somatotroph percentage in the group treated with sulpiride, suggesting an effect of the drug itself on this cell population. Since the antidopaminergic agent/somatotroph cellularity ratio has not been explored in the literature, it is not possible to compare the present data with those of others.

The treatment with estrogen increased the percentage of lactotrophs by approxi-

Table 1 - Percentage of immunoreactive somatotrophs in rat anterior pituitaries, in the absence of treatment and under estrogen or dopamine receptor antagonist treatment.

[a]P<0.05 compared to haloperidol, fluphenazine and metoclopramide (ANOVA). [b]P<0.05 compared to haloperidol and fluphenazine (ANOVA).

Group	N	Somatotrophs (%mean ± SD)	Confidence intervals (95%)	Minimum/maximum (%)
Control	9	27.3 ± 3.8[a]	24.4-30.3	22.6-34.3
Haloperidol	7	23.1 ± 3.0	20.7-25.9	19.1-26.5
Fluphenazine	8	22.1 ± 1.1	21.2-23.1	20.0-23.4
Sulpiride	7	25.0 ± 2.2	22.9-27.1	23.2-28.7
Metoclopramide	9	24.2 ± 3.0	21.9-26.6	18.9-27.7
Estrogen	7	27.1 ± 2.8[b]	24.5-29.8	22.8-31.3

Table 2 - Percentage of immunoreactive lactotrophs in rat anterior pituitaries, in the absence of treatment and under estrogen or dopamine receptor antagonist treatment.

[a]P<0.05 compared to the other groups (ANOVA). [b]P<0.05 compared to fluphenazine, sulpiride and metoclopramide (ANOVA). [c]P<0.05 compared to haloperidol, fluphenazine, sulpiride and metoclopramide (ANOVA).

Group	N	Lactotrophs (%mean ± SD)	Confidence intervals (95%)	Minimum/maximum (%)
Control	9	21.3 ± 4.4[a]	17.9-24.8	16.0-28.0
Haloperidol	7	27.8 ± 2.2[b]	25.8-29.9	25.0-31.0
Fluphenazine	8	34.5 ± 3.6	31.4-37.6	31.0-42.0
Sulpiride	7	32.7 ± 3.5	29.4-35.9	26.0-36.0
Metoclopramide	9	33.4 ± 5.5	29.1-37.7	25.0-40.0
Estrogen	7	42.4 ± 2.8[c]	39.8-45.0	37.0-45.0

mately 100%, while there was no reduction in somatotroph percentage. Several investigators have reported a decrease in the relative number of somatotrophs under treatment with estrogen (3,7-11). In most of these studies, the evaluation was visual and rough and only on two occasions (3,11) was it quantitative or semiquantitative. In contrast to the studies mentioned above and in agreement with our data, Ho et al. (4) observed a hyperplastic effect of estrogen on the somatotrophs and other pituitary cells. According to these investigators, the absolute population of somatotrophs, lactotrophs and non-GH/PRL-producing cells increases in castrated male rats treated with estradiol by an unknown mechanism of action. There are no studies identifying the role of variables such as animal strain, sex and age or the length of treatment in the discrepancy of the data obtained.

In conclusion, the present results suggest a stimulatory action of estrogen on somatotroph replication and raise the possibility of a similar action for drugs that share an antidopaminergic action with estrogen.

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