

Hormone Secretion by Cell Culture of Human GH-PRL Secreting Pituitary Adenomas: Effects of Bromocriptine*

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Summary: Dopamine agonists effectively reduce the secretion of prolactin (PRL) in the great majority of prolactinomas and reduce the bulk of the adenomas, as well as have partial therapeutic effect on some patients with acromegaly. The inhibitory effect of bromocriptine (BC), a dopamine agonist, on growth hormone (GH) and PRL secretion of dispersed cells from the pituitary adenomas of 16 cases of acromegaly, which secrete GH and PRL simultaneously, were evaluated *in vitro*. The significant inhibitory effects of BC on PRL secretion were found in 12 cases. It was also found that PRL secretion was strongly inhibited when GH was suppressed; on the contrary, when GH secretion was not suppressed, the production of PRL was not or weakly inhibited. The exact mechanism of the effects is unclear so far. It is necessary to investigate, at molecular level, the etiology of GH-PRL adenomas and its response to therapeutic agents.

Key words: pituitary adenoma; growth hormone; prolactin; bromocriptine

Recent researches showed that both somatotrophinomas and growth hormone (GH)/prolactin (PRL) releasing adenomas represented acromegaly^[1], but their response to chemotherapy's and prognosis were different^[2]. In this study, the inhibitory effect of bromocriptine (BC), an agonist of dopamine commonly used to treat pituitary adenomas, on GH and PRL secretion of GH-PRL secreting adenomas were evaluated.

1 MATERIALS AND METHODS

Pituitary adenomas of 16 patients with acromegaly were examined. Of the 16 patients, 8 were male and 8 were female. Tumor tissues were taken from the patients who received transsphenoidal or transcranial resection. Preoperative endocrine tests showed that all the patients had high levels of GH and PRL. For cell culture, freshly resected tissues were washed immediately in phosphate-buffer saline containing strepto-

mycin 200 µg/ml, penicillin 200 U/ml, and fungizone 2.5 µg/ml and then cut into small pieces with scalpels. The tissue fragments were incubated at 37 °C with collagenase in an orbital incubator shaker for 2 h. The dispersed cells were washed and re-suspended in MEM containing 10 % fetal calf serum (FCS), non-essential amino acids, 20 mmol/L HEPES, 0.75 % NaHCO₃, 100 µg/L streptomycin, and 100 U/L penicillin [here after referred to as culture medium (CM)]. For each case examined, equal aliquots of pituitary cells were distributed into 12 glass culture tubes and allowed to attach and equilibrate during the next 18 to 24 h, then the cells were washed with CM and further equilibrated at 37 °C for 4–6 h in fresh CM (2 ml). After being washed with renewed MEM (change 10 % FCS to 5 % FCS treated with activated carbon)^[2], the cells were incubated together with fresh MEM with or without 5 µmol/L BC (the used dosage was identified to have maximum effect in cell culture system)^[3], with 3 tubes in each group. After 24 h incubation, the medium was collected and stored at -20 °C for RIA determination of GH and PRL.

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2 RESULTS

The effects of BC (5 $\mu\text{mol/L}$) on GH and PRL secretion of 16 cases of pituitary GH-PRL adenomas in 24 h cell culture were shown in table 1. Among them, 12 cases had significant inhibitory effects of BC on GH secretion, and the remaining 4 had no

statistically significant inhibitory effects. On the contrary, 6 of them had statistically significant inhibitory effects of BC on PRL secretion, and the remaining 10 cases had no statistically significant inhibitory effects. It was also found that there was no or weak inhibitory effect of BC on PRL secretion ($P < 0.05$) in the tumors in which BC has no inhibitory effects on GH secretion.

Table 1. Effect of BC on GH and PRL secretion of 16 cases of pituitary GH-PRL adenomas

Case	Sex	GH secretion (% , $\bar{x} \pm s$)		PRL secretion (% , $\bar{x} \pm s$)	
		Control	BC	Control	BC
1	Male	100 \pm 10	56 \pm 5**	100 \pm 27	34 \pm 4
2	Male	100 \pm 21	76 \pm 32	100 \pm 23	34 \pm 8*
3	Female	100 \pm 12	43 \pm 7***	100 \pm 14	17 \pm 2***
4	Female	100 \pm 7	86 \pm 7	100 \pm 15	73 \pm 4*
5	Male	100 \pm 17	6 \pm 0***	100 \pm 11	11 \pm 4***
6	Female	100 \pm 32	63 \pm 12	100 \pm 24	32 \pm 8
7	Male	100 \pm 5	95 \pm 26	100 \pm 13	55 \pm 25
8	Female	100 \pm 13	23 \pm 3***	100 \pm 4	50 \pm 13**
9	Male	100 \pm 58	70 \pm 22	100 \pm 43	82 \pm 12
10	Male	100 \pm 9	124 \pm 9	100 \pm 48	110 \pm 9
11	Female	100 \pm 8	133 \pm 3	100 \pm 28	24 \pm 20*
12	Male	100 \pm 12	19 \pm 6***	100 \pm 13	15 \pm 0.7***
13	Female	100 \pm 19	75 \pm 15	100 \pm 10	79 \pm 17
14	Male	100 \pm 20	88 \pm 5	100 \pm 61	28 \pm 2
15	Male	100 \pm 50	136 \pm 56	100 \pm 7	63 \pm 10**
16	Female	100 \pm 29	25 \pm 4**	100 \pm 9	21 \pm 0.5***

* $P < 0.05$ ** $P < 0.01$ *** $P < 0.001$, as compared with control

3 DISCUSSION

Recently, the dopamine agonists were mainly used for the treatment of PRL adenomas. Such agents not only reduce the PRL gene transcription and PRL releasing of PRL cells, but also reduce the bulk of the tumors in simple PRL adenomas^[5], and have inhibitory effect on secretion of somatotrophinomas and other pituitary adenomas^[2, 4]. In recent years, a mixed adenoma secreting both GH and PRL was drawing the attention of researchers. It was believed that there were some differences in biochemical features between GH-PRL adenomas and simple GH or PRL adenomas^[2]. GH-PRL adenomas were found to respond

well to dopamine agonists. In this study, BC, a dopamine agonist, showed a similar inhibitory effect on both GH and PRL secretion, suggesting that BC also has inhibitory effect on the releasing of PRL when the GH secretion was suppressed, and the effect of BC on PRL secretion was stronger than that on GH secretion. BC had no or weak inhibitory effect on PRL level when the GH secretion was not suppressed. The mechanism of the interrelation between GH and PRL secretion was still unclear. In general, the secretion of PRL is produced by the inhibitory effect of dopamine-2 receptor via secreting PRL cells. When dopamines combine with receptor, G protein coupled them with intracellular signal transduction system, and further gave rise to biological ef-

fects. When dopamine-2 receptor is activated, intracellular adenylyl cyclase activity mediated by inhibitory G protein and cAMP level were reduced, and the turnover of phosphatidyl inositol, calcium inflow and protein kinase C activity were inhibited^[4,7]. This is similar to the mechanism of intracellular signal transduction of GH adenomas^[8].

For a long time, the difficulty in obtaining samples of resected PRL adenomas, most of them were microadenomas and treated by chemotherapies, hindered the researches on the study of its pathogenesis, the tolerance to pharmaceutical treatment and recurrence at molecular level. The present study was shown some preliminary evidence that dopamine-2 receptor had no gene mutations^[6], the regulation mechanism of postreceptor inhibitory signal transduction systems in these diseases needs further researches, as suggested by many researchers^[6-8].

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