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Bromocriptine Inhibits Growth Hormone Release from Rat Pituitary Cells in Primary Culture (41787)

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Abstract. The action of the potent dopamine receptor agonist bromocriptine was studied in primary cultures of rat anterior pituitary cells. Bromocriptine inhibited both prolactin and growth hormone release in a concentration-dependent manner. This effect was blocked by the dopamine receptor antagonist spiperone when the agonist and antagonist were added coincidently. In contrast, spiperone was unable to affect the actions of bromocriptine if added 1 min after bromocriptine application or later. These results suggest that dopamine receptors exist not only on mammotrophs, but also on somatotrophs *in vitro.*

Studies of dopamine (DA) receptor activity related to prolactin release have predominated over the last decade, although a growing number of reports suggest that the DA receptor phenotype is not exclusive to the mammotroph in the anterior pituitary gland. For example, on the basis of receptor binding and secretory data, Foord and colleagues (1) proposed that the thyrotroph expresses DA receptors that, when activated, initiate the inhibition of thyrotropin release. The possibility of DA receptors on the somatotroph has also been entertained and tested with *in vitro* approaches. The dopaminergic agonist bromocriptine reduced prolactin and growth hormone (GH) release from "normal" human pituitary cells, removed during treatment for breast carcinoma (2). However, the very high bromocriptine concentrations used (i.e., 15 n*M* to 15 μM) produced no dose–response relationship; all concentrations inhibited GH release by about 50%. In rat pituitary cells, a single high concentration of bromocriptine (i.e., 100 n*M*) also significantly reduced both prolactin and GH release (3).

It is essential to define the cellular distribution of DA receptors in order to identify other pituitary cell types potentially under dopaminergic control. Such data would also serve to countermand the assumption that whole-pituitary DA receptor measurements are necessarily representative of the mammotroph. Therefore, we attempted to determine if bromocriptine could indeed inhibit GH release *in vitro* in a concentration-dependent and pharmacologically reversible manner, criteria for a DA receptor interaction that had not been satisfied in the previous studies (2, 3). In the process of this investigation, we found that a potent DA receptor antagonist was ineffective in blocking the effects of bromocriptine if the antagonist was added after bromocriptine. We pursued this unexpected finding to define the minimal time of bromocriptine exposure required to inhibit GH release that could not be affected by subsequent addition of the antagonist.

Materials and Methods. Sprague–Dawley rats (180–220 g, Hilltop Labs, Scottsdale, Pa.) of both sexes were maintained at 21 ± 1°C on a 12:12 light:dark schedule with food and water *ad libitum*. When the animals were sacrificed, the anterior pituitary glands were removed and prepared for primary culture as previously described (4, 5). On the day of the study, the cells were washed 4× in serum-free RPMI-1640 medium with antibiotics (GIBCO, N.Y.) and then the cells were exposed to either vehicle (0.001% EtOH in medium), bromocriptine (gift of Dr. E. Fluckiger, Sandoz, Basel), and/or synthetic human pancreas tumor GH-releasing factor-44 (GRF: gift of Dr. R. Guillemin and Dr. N. Ling, The Salk Institute, La Jolla) for various times. The

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DA receptor antagonist spiperone (gift of Janssen, Breese, Belgium) was added to the medium in some studies either prior to, coincident with, or following the addition of bromocriptine. The incubation proceeded in a 37°C humidified atmosphere of 95% air/5% CO_2 and the reactions were terminated by removing the medium and extracting the cells for either protein (0.2 *N* NaOH, overnight at 0–2°C) or cyclic AMP (0.1 *N* HCl, 37°C, 10 min). Samples were stored at −20°C until assayed.

Protein concentrations were determined by the method of Bradford (6). In the studies that had too little protein to be measured, the data are expressed as hormone released per well. Cyclic AMP levels were assayed according to protocols previously published for the Gammaflow automated radioimmunoassay (7). Rat GH, prolactin and luteinizing hormone were assessed by radioimmunoassay based on protocols and reagents supplied by the National Hormone and Pituitary Agency (NIADDKD anti-rat GH serum-1 and rat GH-RP-1 standard: rat prolactin RP-2 standard: anti-rat LH serum-5 and rat LH-RP-1 standard). The intra- and interassay coefficients of variation were less than 10%. The differences between groups were calculated by an analysis of variance with a Neuman–Keuls test for significance. All results are representative of at least two independent experiments.

Results. Bromocriptine induced a concentration-dependent inhibition of prolactin and GH release (Fig. 1, Table I) and cellular cyclic AMP (31% maximal inhibition, $P < 0.05$), with no effect on basal luteinizing hormone release (control 1140 ± 50 ng LH/well, 50 n*M* bromocriptine 1050 ± 90 ng LH/well, $N = 5$ each). The bromocriptine effect on basal cyclic AMP was inconsistent in the absence of a phosphodiesterase inhibitor, as has been previously noted (8). The degree of maximal inhibition was always greater for prolactin (89 ± 2%, $N = 3$) than GH (59 ± 6%, $N = 7$). This effect on GH release was observed in both male- and female-derived cells and the half-maximal inhibition occurred at less than 1 n*M* bromocriptine (Fig. 1, Table I). The effects of GRF, a specific stimulator of both cyclic AMP accumulation and GH release (4, 5), were also attenuated by bromocriptine (Table II). None of these changes are likely to be due to a change in cell attachment because the average protein concentrations did not differ between groups ($P > 0.05$).

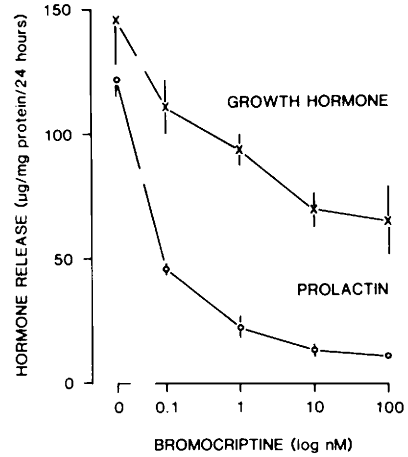

FIG. 1. The concentration dependence of bromocriptine-induced inhibition of GH and prolactin release is illustrated. Primary cultures of anterior pituitary cells from male rats were exposed to the vehicle or bromocriptine for 24 hr. The mean ± SEM is represented, N = 3–5/point. The control GH value is different from the values at 1, 10, and 100 n*M* bromocriptine ($P < 0.05$); the control prolactin value is different from all bromocriptine prolactin levels ($P < 0.05$).

The potent DA receptor antagonist spiperone (9) was capable of blocking the inhibitory action of bromocriptine if and only if the spiperone was added coincident with the bromocriptine (Fig. 2). One minute or more of the unopposed action of bromocriptine was sufficient to produce the full inhibition of GH release (Fig. 2) as measured at 240 min, despite the presence of 10 n*M* spiperone during 180 to 239 min of the experiment. Spiperone alone had little or no effect on hormone secretion (data not shown).

Discussion. A number of investigators have observed that DA agonists reduce the rate of GH release from GH-secreting pituitary adenomas *in vitro* (reviewed in (10)). However, the relevance of this property to normal somatotrophs has been questioned because of the possible expression of ectopic DA receptors on these tumor cells. Data more relevant to the normal condition were recently presented by Hanew and Rennels (3). They showed that a high concentration of bromocriptine (i.e., 100 n*M*) inhibited GH release from normal

TABLE I. BROMOCRIPTINE INHIBITS GH RELEASE FROM SOMATOTROPHS OBTAINED IN PRIMARY CULTURES OF PITUITARY CELLS FROM BOTH FEMALE (EXPT 1) AND MALE (EXPT 2) RATS

	Bromocriptine (n*M*)	GH released (μg/well)
Experiment 1	0	2.94 ± 0.56 (4)*
	0.1	1.45 ± 0.39 (4)
	1.0	1.04 ± 0.39 (4)
	10	0.96 ± 0.13 (4)
	100	0.55 ± 0.20 (3)
	Bromocriptine (n*M*)	GH released (μg/mg protein)
Experiment 2	0	254 ± 16 (5)
	0.01	256 ± 16 (5)
	0.03	220 ± 17 (6)
	0.10	208 ± 20 (5)
	1.0	162 ± 8 (6)*

Note. The cells were incubated ± bromocriptine for 24 hr and the data are represented as the mean ± SEM (*N* wells/group). These experiments were performed on different days.

* Different from all other groups at $P < 0.05$.

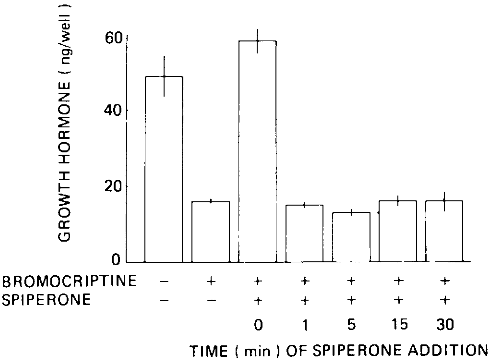

FIG. 2. The inability of spiperone (10 n*M*) to block bromocriptine (50 n*M*) inhibition of GH release is shown. Spiperone was added to the wells either coincidently with bromocriptine (time 0) or 1, 5, 15, or 30 min after bromocriptine. The total incubation time was 4 hr for the cultured cells from female rats (mean ± SEM, *N* = 4/group).

anterior pituitary cells in monolayer culture; this effect was reduced with time in primary culture, from a 30% inhibition at 3 days to approximately a 15% inhibition at 6 and 9 days. This modest level of inhibition, when compared to prolactin, may well explain previous results that did not show decreased GH secretion *in vitro* (11–13).

Immunocytochemical and autoradiographic evidence also supports the possibility of DA receptors on somatotrophs. Immunostaining of DA receptors was noted on 1/3 of all identified somatotrophs, although the degree of staining was never as great as that seen in mammotrophs (14). Furthermore, dopamine itself was internalized by rat somatotrophs in a manner similar to the mammotroph, as defined by quantitative EM autoradiography (15). Both of these studies used enzymatically dispersed pituitary cells from normal rats after a day of suspension culture.

Alternative explanations for these data are more provocative. One possiblity is that a normal pituitary cell type, which may secrete both prolactin and GH (16), or a subclass of somatotrophs (17) may also express the DA receptor phenotype. The cell secreting both hormones *in vitro* (16) is reminiscent of the so-called mammosomatotroph cell adenoma

TABLE II. THE EFFECT OF BROMOCRIPTINE ON GRF-STIMULATED CELLULAR CYCLIC AMP LEVELS AND GH RELEASE

Bromocriptine (n*M*)	Cyclic AMP (pmole/mg protein)		GH release (μg/mg protein)	
	−GRF	+GRF	−GRF	+GRF
0	68.4 ± 4.3	381 ± 38	25.9 ± 2.5	139 ± 16
1	81.5 ± 8.6	282 ± 26	22.7 ± 3.2	120 ± 17
10*	70.8 ± 3.1	253 ± 47	14.5 ± 2.2	95.3 ± 14

Note. The cells were pretreated with the various bromocriptine concentrations for 20 hr prior to washing and addition of fresh bromocriptine and 10 n*M* GRF. The data are represented as means ± SEM, *N* = 5–6/group, for the 4-hr incubation period.

* At this concentration bromocriptine significantly inhibited GRF-stimulated cyclic AMP levels and GH release ($P < 0.05$).

characterized in humans (18). Another conceivable interpretation is that a paracrine event could have caused the inhibition of GH release in these long-term static incubations. There are reductions in extracellular prolactin, thyrotropin, and cyclic AMP (at least) and any of these hormones may be trophic for basal GH release. There is precedence for such a proposal in that a humoral factor secreted by gonadotrophs can activate mammotrophs (19) and prolactin itself can apparently suppress the gonadotroph (20) *in vitro.* Finally, somatotrophs may dedifferentiate in culture to express functional DA receptors or the presence of bromocriptine chronically may induce expression of the DA receptor on the somatotroph. Although the relevance of these data to normal cell physiology of the somatotroph *in vivo* is impossible to determine, these observations should incite, at the least, *in vivo* studies of labeled DA receptors (e.g., autoradiography) associated with immunochemically identified pituitary cell types.

The finding that spiperone cannot antagonize the bromocriptine inhibition of hormone release after 1 min of bromocriptine is intriguing. It has been shown that once the bromocriptine effect is established, it is prolonged for hours in the absence of extracellular free bromocriptine (21). Spiperone itself is one of the most potent DA receptor antagonists known (9) and a concentration of 10 n*M* would be expected to rapidly associate with the DA receptor as judged by binding studies (23). It is also assumed that the inhibitory effects of bromocriptine require the presence of a DA receptor moiety because of data from DA receptor deficient clones of pituitary tumor cells (23–25). Thus, it is possible that bromocriptine must bind to only a small fraction of the free DA receptors to engage the full inhibitory mechanism. Based on this reasoning, even though spiperone may have bound to the majority of the DA receptors if added at 1 min, the blockade of these "spare" receptors would have had no effect on hormone release.

In conclusion, the bromocriptine inhibition of GH release indicates a potential for DA receptors to exist on at least some nonadenomatous somatotrophs *in vitro.* Furthermore, this finding also provides a basis for explaining the responsiveness of some somatotroph adenomas to bromocriptine therapy (reviewed in (10)). Finally, the functional irreversibility of the bromocriptine effects after 1 min of treatment dramatizes the potency of this drug and suggests that there are spare DA receptors on cells of the anterior pituitary gland.

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