THE MODE OF ACTION OF BROMOCRIPTINE

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1. Introduction

Bromocriptine, an ergot alkaloid and dopamine agonist, is the treatment of choice for the induction of ovulation and pregnancy in cases of infertility due to both functional and organic hyperprolactinemia, the latter being due to prolactin secreting pituitary adenomas. It was found that 5–22% of the population harbour pituitary adenomas [1,2]. Bromocriptine has also been useful in the treatment of the symptomatic enlargement of pituitary adenomas during pregnancy as documented by tumor size reduction [3].

Bromocriptine crosses the placenta and enters the fetal circulation reducing prolactin secretion in both maternal and fetal pituitaries [4]. At the level of the pituitary lactotroph there is evidence of the existence of dopamine receptors on the cell membrane and that bromocriptine acts as a dopamine receptor agonist [5]. It has been suggested that bromocriptine acts primarily on prolactin secretion, and that the subsequent accumulation of prolactin within the pituitary inhibits translation and possibly transcription, by an intracellular negative-feedback mechanism [6]. The results of our investigation indicate that bromocriptine itself may act by inhibiting transcription.

2. Materials and methods

L-[4,5-^{3}H]Leucine (spec. act. 30.2 Ci/mmol and [5,6–^{3}H]uridine (spec. act. 40.3 Ci/mmol) were purchased from Kamag, Negev. [^{125}I]Iodine (carrier free) was purchased from Radiochemical Centre, Amersham. Rat prolactin and corresponding antiserum were supplied by the NIAMDD hormone distributors program (Dr Parlow). Bromocriptine (CB-154) was kindly donated by Sandoz, Switzerland.

Pituitaries were surgically removed from 200 ± 20 g Sabra strain female rats and bisected. Each hemipituitary was incubated in Krebs–Ringer–bicarbonate buffer at 37°C with 10 μCi [^{3}H]leucine or 15 μCi [^{3}H]uridine with or without bromocriptine (3×10^{-5} M). At each time indicated, the tissue was separated from the medium, homogenized in 0.25 M sucrose TKM buffer (Tris–HCl (pH 7.4) 50 mM; KCl 25 mM; $MgCl_2$ 10 mM) and spun in the presence of 1% Triton X-100; 1% deoxycholate mixture at $1000 \times g$ for 20 min. The supernatants (tissue) and the media were analyzed.

The incorporation of radioactive tracer was measured as in [15]. Protein content was determined as in [16]. Prolactin radioimmunoassay was done according to the protocol supplied by the NIAMDD hormone distributors program. SDS gel electrophoresis was done as in [10].

Samples were run on a 15% polyacrylamide SDS gel. Prolactin bands were identified, excised and dissolved in H_2O_2:SDS (24%:2%) 1:1 (v/v) and counted in a liquid scintillation counter. Isolation of poly [A(–)] and poly [A(+)] RNA was done as in [8].

3. Results and discussion

Fig.1 shows the effect of bromocriptine on the synthesis (fig.1A) and secretion (fig.1B) of radioactive proteins by the rat hemipituitaries.

At 3×10^{-5} M, bromocriptine causes a 50% inhibition of incorporation of leucine into total protein from the first hour (whereas a dosage of 1×10^{-5} M gives to a 20% inhibition of synthesis and secretion of radioactive proteins, illustrating a clear dose–response effect).

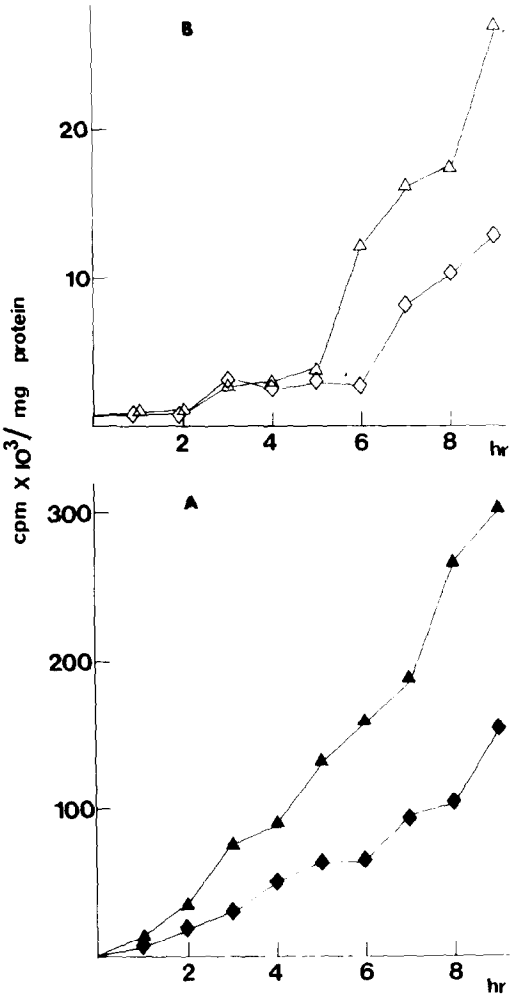

Fig.1. The effect of bromocriptine on the incorporation of [^{3}H]leucine into total tissue proteins (A), and on the secretion of radioactive proteins into the medium (B): control (▲,△); bromocriptine (◆,◇).

Up to 5 h, there is no secretion of radioactive proteins into the medium. At 5–6 h, the control hemipituitaries begin to secrete radioactive proteins in a nearly linear mode. From 6–8 h, secretion occurs in bromocriptine-treated hemipituitaries at the same rate as in the controls, but after 9 h, 50% inhibition of secretion of radioactive proteins is visible in the medium of treated pituitaries. The effect of bromocriptine on the synthesis and secretion of newly synthesized prolactin was investigated (fig.2).

In the tissue of the control hemipituitaries the synthesis of radioactive prolactin increases steadily up to 6 h, when a sharp acceleration in synthesis is evident. In the bromocriptine-treated tissue, inhibition is apparent from 1 h, reaching a maximum of 70% at 10 h incubation. The first molecules of radioactive prolactin do not appear in the medium of the control hemipituitaries until after 6 h, since this is the time period required for the synthesis, transport and packaging of the hormone prior to secretion [7]. Although

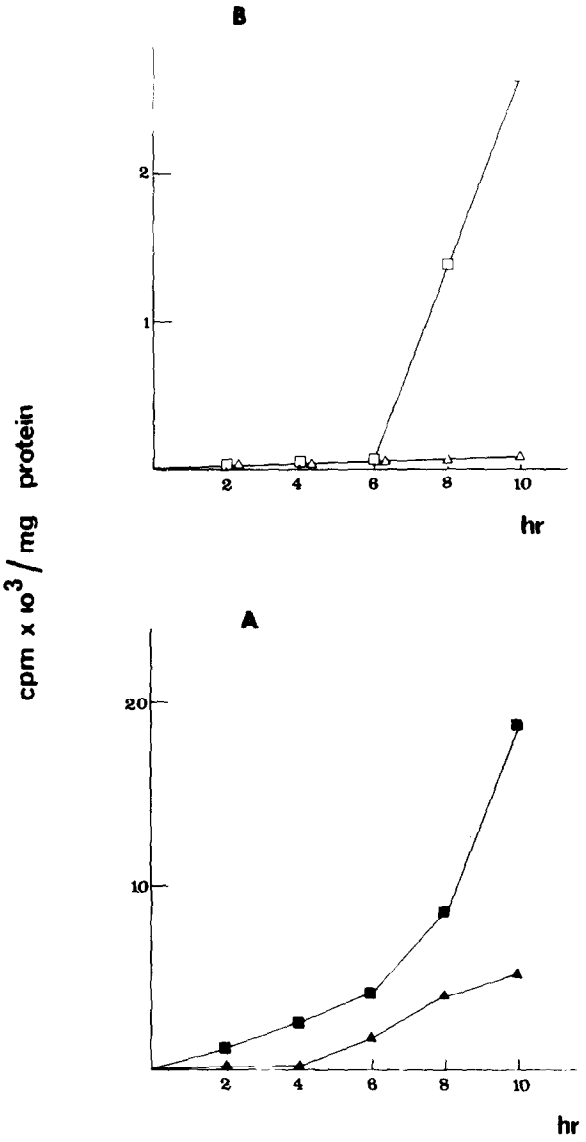

Fig.2. The effect of bromocriptine on the incorporation of [^{3}H]leucine into tissue prolactin (A) and secreted labelled prolactin (B): control (■,□); bromocriptine (▲,△).

total inhibition of synthesis does not occur in the tissue, in the medium, bromocriptine halts completely the secretion of the de novo synthesized prolactin. Double antibody radioimmunoassay of the total prolactin content in both the tissue and the medium showed that in the tissue there was a sharp decrease in the amount of prolactin present in the first 2 h and this was accompanied by a corresponding increase in the medium. At zero time, no prolactin was measured in the medium, and no prolactin band was present in the gel.

These observations led us to conclude that bromocriptine had no effect on the secretion of the pre-existing pool of prolactin, and only acted on the newly synthesized hormone.

With these results, it became necessary to investigate the possible effects of the drug on RNA synthesis, to determine whether transcription or translation was the primary site of action.

Fig.3 shows the effect of bromocriptine on the incorporation of uridine into RNA. In the untreated control hemipituitaries, RNA synthesis begins immediately, increasing gradually up to 8 h, where a sharp acceleration of the rate of synthesis is evident.

In bromocriptine treated hemipituitaries, inhibition of synthesis is seen from 1 h until the end of the incubation, where a maximum of 80% inhibition is reached. No radioactivity was detected in the medium, demonstrating the integrity of the tissue during the incubation.

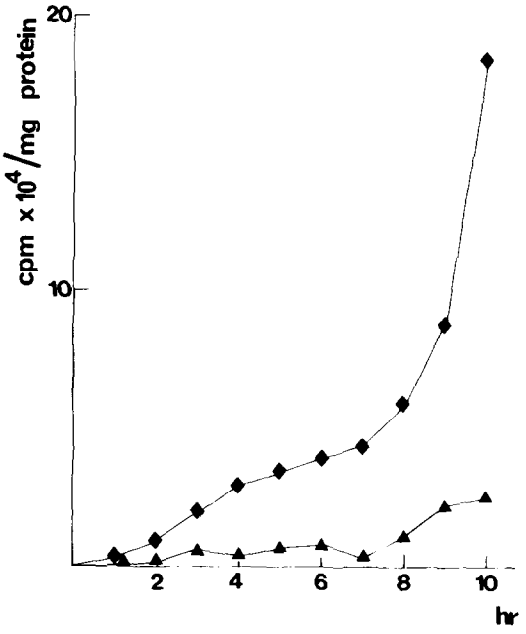

Fig.3. The effect of bromocriptine on the incorporation of [³H]uridine into total RNA: control (♦); bromocriptine (▲).

Table 1
The effect of bromocriptine on poly[A(−)] and poly[A(+)] RNA synthesis

	Poly[A(−)] RNA	Poly[A(+)] RNA
(A) Control	10 300	5400
Bromocriptine	1670	1640
(B) Control, 4 h	35 400	116 400
Bromocriptine 4 h	14 700	19 300

Poly[A(−)] and poly[A(+)] RNA fractions were isolated as in [8]. (A) Hemipituitaries were incubated for 10 h in the presence of 15 μCi [³H]uridine/sample. All samples were pooled and subjected for isolation of RNA. (B) Hemipituitaries were incubated for 4 h, then treated as above. Incubation medium contained 20 μCi [³H]uridine/sample. The results are expressed as cpm/A_{260} units isolated RNA.

All the samples represented in fig.3 were pooled and passed over a column of oligo(dT)-cellulose [8], in order to determine whether the inhibition was mRNA-specific or affected total RNA synthesis. In the poly[A(−)] RNA, 84% inhibition was found, while 70% inhibition was seen in the poly[A(+)] RNA fraction (table 1). Hemipituitaries were incubated in the presence of labeled uridine and the RNA was isolated after 4 h. Inhibition of 60% was found in the poly[A(−)] RNA and inhibition of 85% in the poly[A(+)] RNA fraction. It should be noted that the total amounts of RNA present in the controls and the bromocriptine-treated tissue were approximately equal, which shows that the results were due to a specific inhibition of RNA synthesis, and not due to RNase activity.

In order to prove that the poly[A(+)] RNA was in fact mRNA, the isolated fraction was translated in a reticulocyte lysate cell free system [9].

The translation products were run on a SDS–polyacrylamide gel [10] and autoradiographed, and since a major band present co-migrated with an ^{125}I-labeled prolactin marker, and had app. M_r 22 000, a further sample of the translation product was immunoprecipitated with prolactin specific antiserum, rerun on the gel and autoradiographed (fig.4). Only one band was present and co-migrated with the iodinated standard prolactin marker.

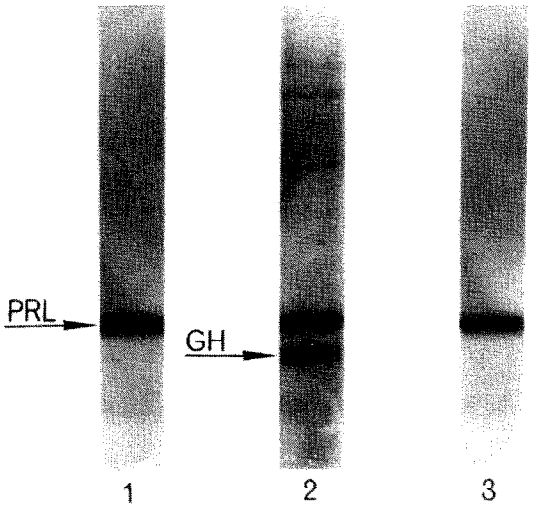

Fig.4. Autoradiogram of translation products in a reticulocyte lysate cell free system: (1) ^{125}I-labeled prolactin marker; (2) translation products; (3) immunoprecipitate of translation products.

Thus, bromocriptine in this system acts only on the secretion of de novo synthesized prolactin in the hemipituitary and not on the pre-existing pool. Serum prolactin takes 1–3 days to reach normal levels when using bromocriptine in hyperprolactinemic patients [11].

Bromocriptine inhibition is much more profound in the poly[A(+)] RNA synthesis (85%) than in protein synthesis (50%) which lends support to the idea that transcription is the primary site of action.

This may explain why bromocriptine is so effective where there is a large production of mRNA in pituitary cells as in the hyperprolactinemic state during pregnancy, or in prolactin secreting adenomas (especially when they are active during pregnancy). This effect of bromocriptine on mRNA may also explain the capacity of bromocriptine to reduce the tumor size of pituitary adenomas [12]. Bromocriptine specifically inhibits prolactin synthesis and secretion and to a lesser degree other pituitary hormones such as growth hormone. It is known that the effect of bromocriptine in patients with growth hormone secreting pituitary adenomas is less effective than with prolactin secreting adenomas [13], or in the mixed type of adenoma [14].

From these results, we propose that the primary effect of bromocriptine is on the transcriptional level.

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