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Effects of *in vivo* and *in vitro* Exposure to Bromocryptine on Testicular LH Receptors and *in vitro* Testosterone Production in Syrian Hamsters

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The effects of *in vivo* and *in vitro* exposure to bromocryptine (CB-154) were studied in testes of Syrian hamsters. In animals treated for two days with CB-154, a decrease in LH receptors (LH-R) was observed, with a greater decrease being measured in hamsters treated for 14 days, when compared with controls. Injection of HCG caused, in hamsters treated with CB-154 for 14 days, up-regulation of LH-R and increased testosterone synthesis in response to HCG administration *in vitro*. These changes were not observed in the two other groups of animals. When testis fragments were incubated with CB-154, those incubated with a large dose (10 μg/ml) had a normal pattern of response to HCG, and those incubated with a small dose (1 ng/ml) had a smaller maximum response. These actions are similar to those observed in men treated with CB-154. It can be therefore concluded that: a) CB-154 has a direct effect on the testes; b) it probably is through modulation of LH-R synthesis; c) Syrian hamsters probably represent the best model for the study of the effects of CB-154 on the testes; and d) the possibility of using CB-154 as an adjuvant of gonadotropin treatment in hypogonadism has to be considered.

Key words: Bromocryptine, Testes, LH receptors, Testosterone, Prolactin, HCG, Hamsters.

Bromocryptine (CB-154; 2-Br-α-ergocryptine), a synthetic analog of ergot alkaloids, is a dopamine agonist used in endocrinological studies for the suppression of prolactin (10). A common observation

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in hyperprolactinemic and normal men treated with bromocryptine is the elevation in circulating LH and testosterone levels (19, 32, 35, 38). Also, bromocryptine is useful in reducing growth hormone levels in acromegalic patients. However, recently it has been shown that bromocryptine has other effects besides inhibiting the release of prolactin and growth hormone.

As was already mentioned, when hyperprolactinemic men are treated with bromocryptine, circulating LH and testosterone (T) levels gradually increase. Similar effects were noted in bromocryptine-injected male rats and mice (24). In rats, incubation with bromocryptine was first reported to decrease basal and gonadotropin-stimulated testosterone production in adult animals (6, 7, 11, 34), and immature rats (16, 18, 31). Moreover, bromocryptine administration *in vivo* to adult rats was reported to reduce LH-R and testicular testosterone levels, as well as the *in vivo* sensitivity of testosterone production to HCG (11). In other reports, although LH-R levels were reduced by bromocryptine, testicular testosterone levels were unchanged (14, 15, 36). Furthermore, bromocryptine did not affect HCG stimulation of testosterone production *in vitro* (14, 36). In studies conducted in mice, incubation with bromocryptine stimulated testosterone production (5), and in mice treated *in vivo* with bromocryptine, this drug did not affect basal or HCG-stimulated plasma testosterone levels. Moreover, although bromocryptine did not have an effect on basal LH-R levels, it did prevent the HCG-induced negative autoregulation of LH-R (1). In immature animals, discordant results were also observed. Whereas in rats bromocryptine may or may not decrease LH-R levels (15, 16), in Syrian hamsters LH-R levels were decreased (30).

Dopamine acts on cells through at least two types of receptors, D_1 and D_2, but there might be more (4, 12, 25, 26, 27). By binding to D_1, as in the parathyroid, it increases cAMP, but when dopamine binds to D_2, as in lactotrophs, it stimulates guanylate cyclase and inhibits adenylate cyclase. In order to try to elucidate the direct or indirect prolactin-non dependent influence(s) of bromocryptine on testicular function, we have studied the *in vivo* and *in vitro* effects of this drug on the testes of Syrian hamsters. The study of a third species should help elucidate the controversies arising from rat versus mouse experiments.

Materials and Methods

Animals. — Adult (> 3 month old) male Syrian hamsters (Lak: LVG (SYR)) were obtained from Charles River. All animals were kept in a room with controlled temperature (20 ± 2 °C) and illumination (16 h light/24 h), with free access to food and water.

In vivo *exposure to bromocryptine.* — Forty-eight hamsters were divided into three groups. One group was injected with sesame oil for 14 days, another group received oil for 12 days and 600 µg of bromocryptine/day for two more days, and the last group received 600 µg/day of bromocryptine for 14 days. Half of the animals in each group received 0.3 IU HCG/g BW, 24 h before sacrifice, and the other half received isotonic saline. At sacrifice, testes were decapsulated, weighed, and a fragment was frozen rapidly using a solid CO_2: acetone mixture, and stored at −70 °C until assayed for LH-R. Six hamsters from each group that received saline were used for testes incubation. Four testes fragments of similar weight, from each of these animals, were incubated with either 0, 3.125, 12.5 or 50 mIU HCG/ml media, in Krebs-Ringer bicarbonate buffer for 4 h at 32 ± 1 °C (8, 33, 37). Media was stored frozen at −20 °C until assayed for testosterone levels.

In vitro *exposure to bromocryptine.* — Testes fragments from 6 hamsters were incubated in Krebs-Ringer bicarbonate buffer at 32 ± 1 °C for 4 h, in the presence of 1 ng, 10 μg or no bromocryptine/ml media, in combination with one of the following doses of HCG: 0, 3.125, 12.5 or 50 mIU/ml media (8, 33, 37). Media was stored frozen at −20 °C until assayed for testosterone levels.

Measurement of LH-R and hormones. — The levels of testicular LH-R were measured by radioreceptoassay as described previously (17). The ^{125}I-HCG used (CR-121) was iodinated by the chloramine-T method, and had an average specific activity of 42.0 μCI/μg and an average maximum binding ability of 42.1 %. The protein concentration in the testicular membrane preparations used for measurement of LH-R was determined by a modification of Lowry's method (20).

Media testosterone was measured by radioimmunoassay using procedures previously described (37). The intra- and interassay coefficients of variation were 8 % and 15 %, respectively, with a sensitivity of 50 pg/ml.

Prolactin was measured using an homologous radioimmunoassay described before (28). The intra-assay coefficient of variation was 6 % and the sensitivity was 0.5 ng/ml.

Statistics. — Data for the LH-R were obtained using the RRAPLOT and RRADOSE programs (2). All data were evaluated utilizing the SPSS-X software package on an IBM mainframe, and using one- or two-way analysis of variance (21-23). Data were tested for normality of distribution by the Kolmogorov-Smirnov test, and for homogeneity of variance by Barlett's test, and log- or square-root transformed as required (23, 29).

Results

Treatment with bromocryptine for either two or 14 days did not affect testes weight in comparison with oil-treated controls (data not shown). As expected, treatment with bromocryptine reduced, very rapidly, plasma prolactin to almost undetectable levels (fig. 1A). Hamsters injected with oil or for two days with bromocryptine had similar patterns of testicular *in vitro* testosterone production in response to different doses of HCG, with levels tending to be lower in animals treat-

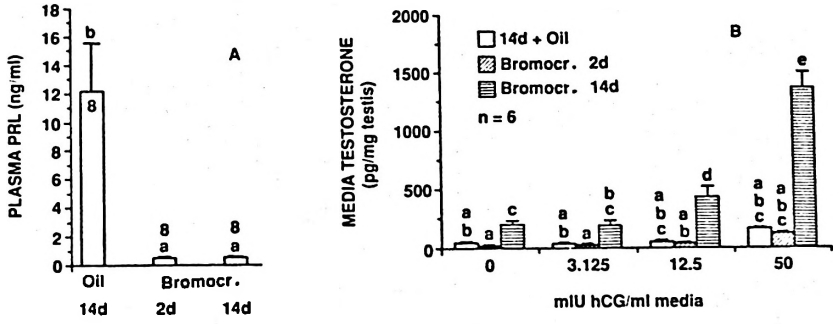

Fig. 1. *Plasma prolactin (A), and incubation media testosterone (B) as a function of the length of treatment with bromocryptine (600 μg/day).*
Values are expressed as mean ± sem for the number of hamsters indicated. Points with the same letter in superscript are not statistically different (P > 0.05; Student-Newman-Keuls procedure of the multiple range test).

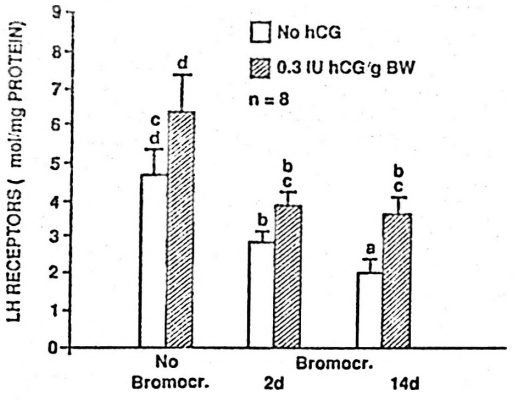

Fig. 2. *Testicular LH receptor concentration as a function of the length of treatment with bromocryptine (600 µg/day), and the dose of hCG.* Values and symbols as in figure 1.

ed for two days with bromocryptine (fig. 1B). However, hamsters which were injected for 14 days with bromocryptine, had significantly higher basal media testosterone levels, and their steroidogenic response to HCG was much more dramatic, when compared to that of animals of the two other treatment groups (fig. 1B). Hamsters injected for two days with bromocryptine had significantly lower

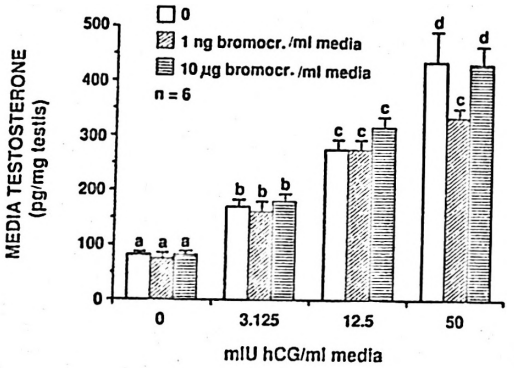

Fig. 3. *Incubation media testosterone as a function of the dose of bromocryptine added to the media.* Values and symbols as in figure 1.

levels of LH-R than controls. Animals treated for 14 days had significantly lower LH-R levels than those of hamsters in the two other groups (fig. 2). Treatment with 0.3 IU HCG/g BW caused, 24 h later, an increase in LH-R levels, however, only in hamsters that received bromocryptine for 14 days was the increase significant (figure 2).

Incubation with either dose of bromocryptine did not affect the levels of testicular LH-R or the basal media testosterone concentration. However, the low dose (1 ng bromocryptine/ml media) reduced the maximal steroidogenic response to HCG, whereas the high dose (10 µg/ml) did not affect the maximal response (figure 3).

Discussion

Because bromocryptine is widely used to treat hyperprolactinemia, and thus restore gonadal function, there has been great interest in studying its effects at each level of the pituitary-testicular axis. However, animal studies with bromocryptine have produced controversial findings with different groups reporting opposite effects of this drug on the testes.

In the present study, bromocryptine administration which causes a duration of treatment-dependent increase in sensitivity to *in vitro* administration of HCG, in basal incubation media testosterone levels, and a decrease in basal LH-R levels, together with the fact that the bromocryptine-induced increase in the sensitivity to HCG allowed *in vivo* HCG to maintain LH-R at control levels, strongly favor a direct action of bromocryptine on Leydig cells. This effect appears to be mediated by bromocryptine potentiating the HCG-dependent LH-R synthesis. Consequently, subsequent administration of HCG can cause a greater stimulation of testosterone production. The decrease in basal LH-R levels, resulting from bromocryp-

tine administration, is probably due to the suppression of prolactin release and loss of the strong positive heteroregulatory action of prolactin on LH-R. However, bromocryptine could also exert other non-LH-R mediated effects as evidenced by the increase in basal media testosterone levels in hamsters treated for 14 days. Short-term exposure to bromocryptine, as in the incubation with the drug, did not affect LH-R levels. Thus, in hamsters, bromocryptine tends to have a positive effect on Leydig cell function.

The present results agree with previous reports that bromocryptine has direct effects on testicular function (19). However, these effects are definitely species-specific. This is not surprising since both the autoregulation of LH-R, and the regulation of testicular steroidogenesis have been shown to be species specific (3). Thus, factors affecting either mechanisms, might induce different species-specific responses. It also appears that the action of bromocryptine on the testes could take place, at least in part, through regulation of the metabolism of LH-R. It has been reported that treatment with bromocryptine tends to stimulate basal and gonadotropin-dependent androgen production, and to reduce LH-R levels, in hyperprolactinemic as well as in euprolactinemic men (13, 19, 32, 35, 38). Since these changes are similar to those observed in Syrian hamsters in the present study, it can be concluded that these rodents, rather than rats or mice, can serve as a better model for the assessment of the effects of bromocryptine on the human testes. By which mechanism(s) bromocryptine would exert its effects directly on the testis is a still unanswered question. One problem has been the various classifications that have been proposed for dopamine receptors, just in the last ten years. SEEMAN proposed the existence of five action sites (25, 26). Others have later suggested that the classification should include at least two types of traditional or central dopamine receptors and two types of peripheral dopamine receptors (4, 12). Since the proposed peripheral D_2 or DA_2 receptors are supposed to be similar to the traditional or central D_2 receptors, bromocryptine should act through them. However, the central and peripheral actions mediated by D_2 receptors are inhibitory, and thus do not fit our observations. Review of all bromocryptine interactions reveals that it can stimulate peripheral D_1 (DA_1) sites, which mediate stimulatory actions, at doses ranging from 110 to 9000 nM, depending on the type of cell (26, 27). For comparison the range for dopamine is of 130 to 70,000 nM. Thus, it is possible that testicular Leydig cells possess a peripheral D_1 receptor that has high affinity for bromocryptine, therefore permitting it to exert the type of actions described herein. Finally, the possible use of bromocryptine as an adjuvant of gonadotropin treatment in primary hypogonadism has to be considered.

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Resumen

Se estudian los efectos *in vivo* e *in vitro* de la bromocriptina (CB-154) en hamster sirios. La administración durante dos días produce disminución en los receptores a LH (LH-R), siendo este efecto mayor en animales tratados durante 14 días. La inyección de HCG produce en estos últimos una autorregulación positiva de los LH-R, así como aumento en la síntesis de testosterona dependiente de HCG *in vitro*. El patrón de respuesta de la testoste-

rona a HCG *in vitro* es similar en los testículos control y en los incubados con una dosis grande de CB-154 (10 µg/ml); en los incubados con una dosis pequeña (1 ng/ml) la respuesta máxima a HCG *in vitro* fue menor. Los efectos de la CB-154 aquí expuestos son similares a los obtenidos en hombres. Se puede concluir que la CB-154 actúa directamente sobre los testículos; que su acción probablemente es debida a la modulación de la síntesis de los LH-R; que los hamsters sirios son probablemente el mejor modelo para estudiar los efectos testiculares de la CB-154; y que debe ser ponderada la posibilidad de usar la CB-154 como adyuvante del tratamiento del hipogonadismo con gonadotropinas.

Palabras clave: Bromocriptina, Testículos, Receptores a LH, Testosterona, Prolactina, HCG, Hamsters.

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