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Possible Role of Prolactin in the Induction of Hypogonadism by Chronic Alcohol Treatment in the Male Rat

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FERMOSO, J , A I ESQUIFINO, A MATEOS, C AGRASAL AND I MARTIN *Possible role of prolactin in the induction of hypogonadism by chronic alcohol treatment in the male rat* PHARMACOL BIOCHEM BEHAV **29**(3) 489–493, 1988 —Treatment of adult male rats with ethanol for a period of six weeks resulted in a numerical but not significant increase in plasma prolactin levels together with a reduction in plasma luteinizing hormone (LH) levels Although basal plasma testosterone (T) levels were not affected in ethanol-treated animals, testicular weight was reduced and seminiferous tubules exhibited signs of atrophy The responses of LH to luteinizing hormone releasing hormone (LHRH) and T to hCG were significantly impaired in ethanol-treated rats ($p<0$ 01) Treatment with bromocriptine (1 mg/kg body weight/day), resulted in the expected decrease in plasma levels of prolactin and an increase in basal plasma LH levels to the levels found in control groups Basal plasma T levels were not affected by bromocriptine However, both plasma LH responses to LHRH and plasma T responses to hCG were significantly improved by bromocriptine treatment in alcoholic rats and became similar to the responses measured in control animals The results suggest that bromocriptine-induced suppression of prolactin release has a beneficial effect on ethanol-induced hypogonadism

Prolactin LH Testosterone Hypogonadism Ethanol intoxication

IT is well established that chronic alcohol intake affects the endocrine system [5–7, 30] Hypogonadism is a common consequence of chronic alcohol abuse in both sexes [5, 19, 28] It is associated with reductions in peripheral LH and testosterone concentrations and a concomitant increase in plasma prolactin levels [6, 10, 13, 22, 23, 28] These findings resemble those encountered in hyperprolactinemia of various etiologies [4, 12, 25, 26] and thus raise a possibility that increased plasma prolactin levels could be responsible for the suppressive effects of alcohol on gonadal function [24]

This work was undertaken to determine whether prolactin may be involved in mediating the effects of chronic alcohol intake on testicular function in adult male rats

METHOD

Adult male Wistar rats (2–3 months old) were purchased from Panlab (Barcelona, Spain) and randomly divided into the following three groups of 60 animals each (1) Alcoholic animals were fed an ethanol-liquid diet (5% w/v) in which ethanol provided 36% of the calories as described in our earlier report [15], (2) pair-fed controls were given a similar diet, except that maltose-dextrine replaced ethanol isocalorically, (3) controls were fed laboratory rat chow and tap water ad lib Animals were given these 3 diets for 6 weeks before the beginning of bromocriptine treatment Twenty mg of bromocriptine (CB-154, Sandoz Pharmaceutical, Basel, Switzerland) were dissolved in 1 ml of absolute ethanol containing tartaric acid, using light flame heating Thirty-nine ml of 0 9% saline were added to obtain a final concentration of 0 5 mg CB-154/ml, pH=5 Alcoholic, pair-fed and control rats received 1 mg of CB-154/kg body weight/day Two-thirds of this amount was given in two equal IP injections at 10 00 and 18 00 hours and the rest was given in the drinking water (10 mg/l prepared as described above except for using water instead of saline) Treatment was followed for 8 days

LHRH Test

Blood for plasma LH measurements was obtained by decapitation under basal conditions or 30 minutes after IP administration of 1 μg of synthetic LHRH (Luforan, Pevya) in 0 2 ml of saline, as in previous studies from this laboratory [14,25] LHRH challenge was performed both before and during bromocriptine treatment (on day 8)

hCG Test

Blood for measurements of plasma testosterone levels (T) was obtained by decapitation under basal conditions at

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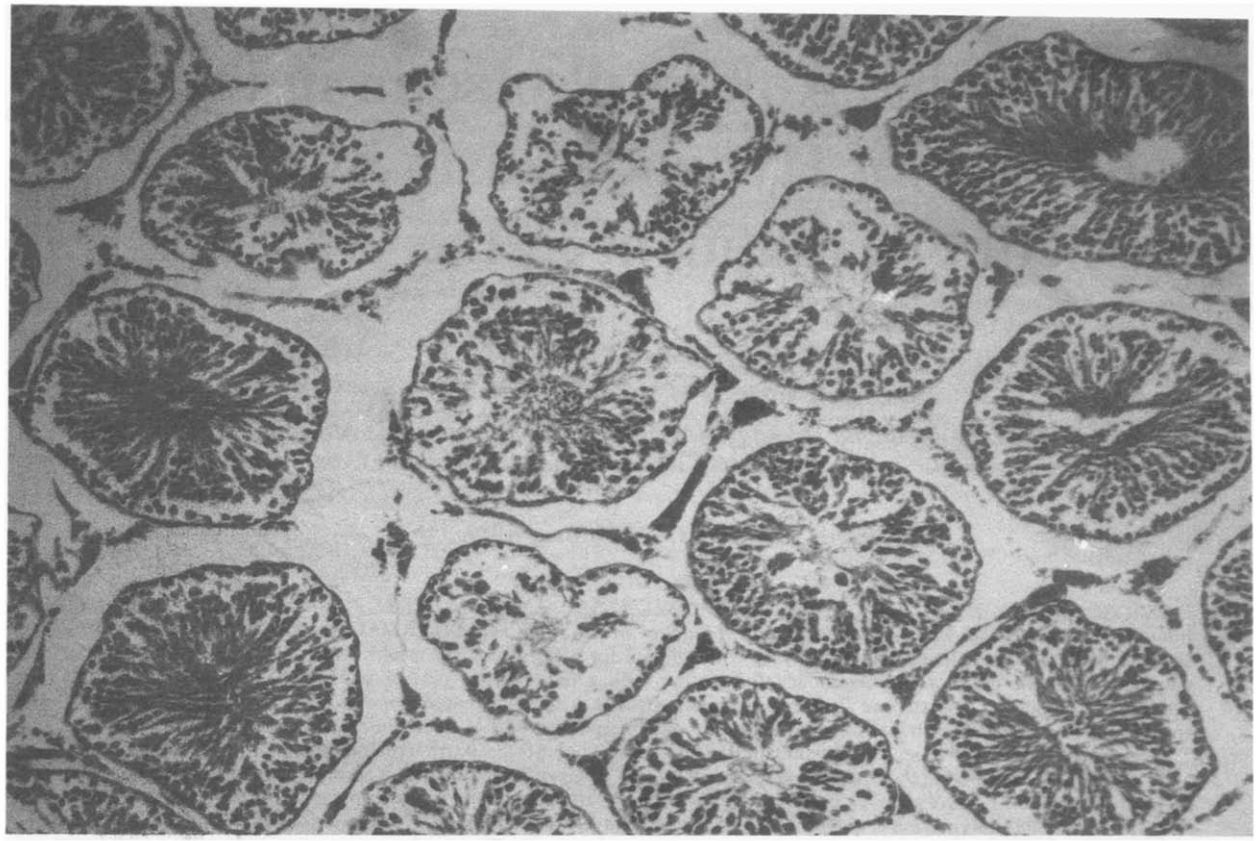

FIG 1 Cross-section of the testis from alcoholic male rats

120 minutes after IV administration of 25 I U of hCG (Physex, Leo), under very light ether anesthesia as in previous studies from this laboratory [26,27] The hCG test was performed both before and during bromocriptine administration (on day 8 of treatment)

Hormone Measurements

The animals were killed by decapitation Plasma from the

RIA systems, previously validated in our laboratory, by using materials kindly supplied by NIH The sensitivity and precision of these methods have been described previously [26] Plasma T levels were measured using a specific commercial kit provided by Sorin Biomedica (Saluggia, Vercelli, Italy), previously validated in our laboratory The sensitivity of the assay was 0 1 ng/ml and the intraassay variation coefficient was 9% To avoid interassay variations all the samples were measured in the same assay

Statistics

Statistical analysis of the data was performed using the Mann-Whitney "U" test

RESULTS

Testicular weight was significantly reduced in alcoholic rats (3 1±0 07 g, $p<0$ 01) as compared to both pair-fed (3 6±0 1 g) and rat chow (3 7±0 1 g) control groups This was accompanied by atrophy of the seminiferous tubules (Fig 1)

Plasma prolactin levels before and after 8 days of bromocriptine treatment are shown in Fig 2 After 6 weeks of continuous alcohol ingestion, alcoholic males exhibited numerically, but not statistically higher plasma prolactin levels when compared to controls (both pair-fed and rat chow groups) Bromocriptine treatment resulted in a signifi-

prolactin concentrations ($p<0$ 01)

After 6 weeks of alcohol ingestion, basal plasma LH levels were significantly reduced ($p<0$ 05) in alcoholic male rats when compared to both control groups (Fig 3) Moreover, plasma LH response to LHRH administration was significantly impaired ($p<0$ 01) in chronic alcoholic male rats as compared to control groups On day 8 of bromocriptine treatment, basal plasma LH levels in ethanol fed rats were increased ($p<0$ 01) and resembled those measured in untreated or bromocriptine-treated control animals The LH response to LHRH in alcoholic rats was also significantly improved after bromocriptine treatment

Basal plasma T levels in chronic alcoholic male rats were similar to those measured in the control groups (Fig 4) whereas their T response to hCG challenge was significantly decreased ($p<0$ 01) On day 8 of bromocriptine treatment there were no differences in basal plasma T levels among any of the groups In alcohol-treated rats, bromocriptine treatment restored the normal T response to hCG

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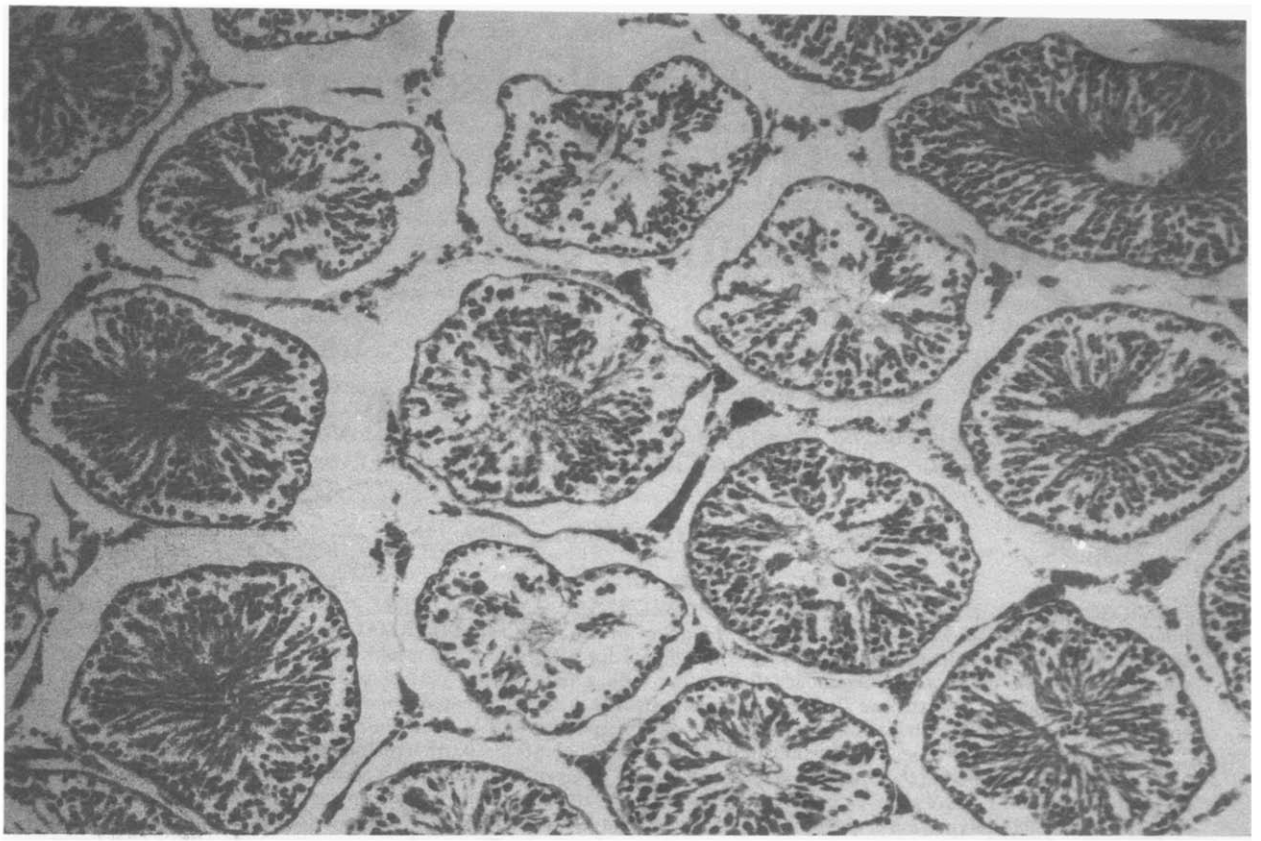

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The animals were killed by decapitation Plasma from the trunk blood was obtained by centrifugation (1,500×g) at 4°C and kept frozen at −20°C until analyzed Plasma prolactin and LH levels were measured by specific double antibody RIA systems, previously validated in our laboratory, by using materials kindly supplied by NIH The sensitivity and precision of these methods have been described previously [26] Plasma T levels were measured using a specific commercial kit provided by Sorin Biomedica (Saluggia, Vercelli, Italy), previously validated in our laboratory The sensitivity of the assay was 0 1 ng/ml and the intraassay variation coefficient was 9% To avoid interassay variations all the samples were measured in the same assay

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Plasma prolactin levels before and after 8 days of bromocriptine treatment are shown in Fig 2 After 6 weeks of continuous alcohol ingestion, alcoholic males exhibited numerically, but not statistically higher plasma prolactin levels when compared to controls (both pair-fed and rat chow groups) Bromocriptine treatment resulted in a significant decrease in plasma prolactin levels in all studied groups as compared to their respective pretreatment plasma prolactin concentrations ($p<0$ 01)

After 6 weeks of alcohol ingestion, basal plasma LH levels were significantly reduced ($p<0$ 05) in alcoholic male rats when compared to both control groups (Fig 3) Moreover, plasma LH response to LHRH administration was significantly impaired ($p<0$ 01) in chronic alcoholic male rats as compared to control groups On day 8 of bromocriptine treatment, basal plasma LH levels in ethanol fed rats were increased ($p<0$ 01) and resembled those measured in untreated or bromocriptine-treated control animals The LH response to LHRH in alcoholic rats was also significantly improved after bromocriptine treatment

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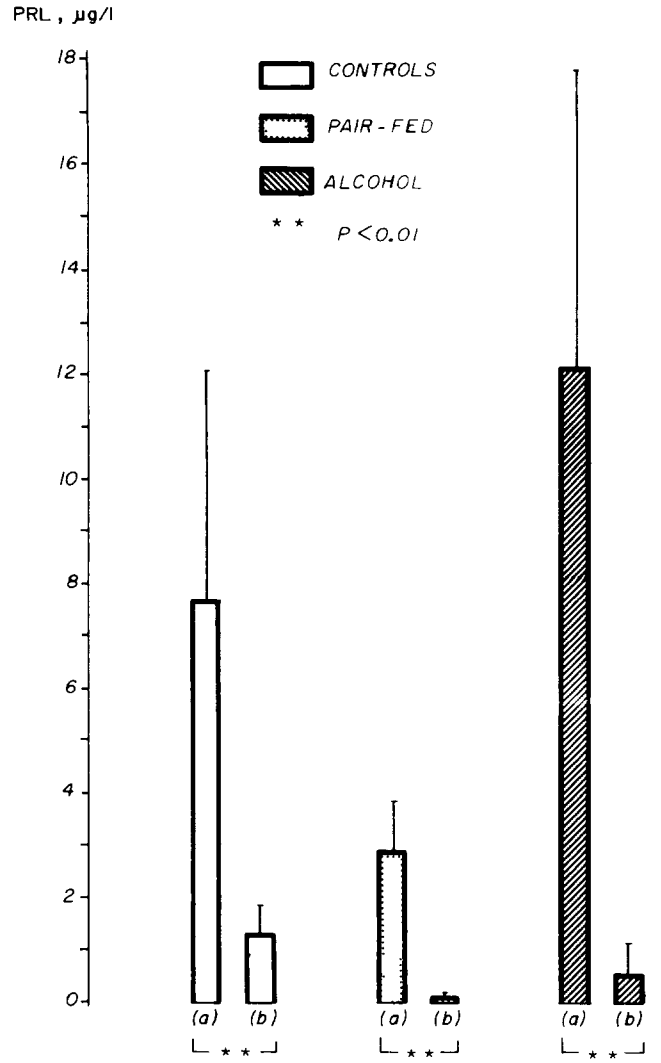

FIG 2 Plasma prolactin levels both basally (a) and during bromocriptine treatment (8th day, b) in alcoholic, pair-fed and rat chow control groups Values are expressed as mean±SD (n=10 animals per group)

DISCUSSION

Our data show a trend towards higher plasma prolactin levels in alcoholic male rats, together with decreased plasma LH concentrations, as has previously been described by other authors [5,21] No differences in basal plasma T levels in ethanol-treated rats could be detected, in contrast to other reports which showed a decrease in plasma T levels [18, 23, 28] The responses of plasma LH to LHRH and plasma T to hCG were decreased in agreement with reports from other laboratories [5, 7, 11]

Interrelationships between prolactin and gonadal function under ethanol intoxication are similar to those in several experimental models of hyperprolactinemia [3,25] It has been reported that normalization of plasma prolactin levels by treatment with a dopamine agonist restored the gonadal function in men [16], but the effects of such treatment in animals with experimental hyperprolactinemia remain controversial [12] As expected, bromocriptine treatment decreased plasma prolactin in both alcoholic and control rats In alcohol-treated animals, these modifications were associated with an increase in plasma LH, LH responses to LHRH, and T responses to hCG, thus suggesting that a slight (non-significant) increase in prolactin levels was sufficient to inhibit the hypothalamic-hypophyseal-gonadal axis This effect appears to have been exerted at the hypothalamic level because the decrease in the LH response to LHRH observed in alcoholic rats before bromocriptine treatment can probably be ascribed to chronic suppression of endogenous LHRH release The existence of this mechanism of prolactin action on LH release has been suggested previously on the basis of studies in other models of hyperprolactinemia [14,31]

However, a direct effect of alcohol on hypothalamic neurotransmission cannot be excluded [20] and both mechanisms could be operating synergistically Other possible mechanisms of suppression of testicular function in alcoholic rats could include direct effects of prolactin and ethanol on the testis, the former through the existence of prolactin receptors in the Leydig cells [1,27] and the latter by acting directly on these cells [28]

No differences in mean basal T levels were detected in ethanol-treated rats, in contrast to previous work [5–7] in which decreased plasma T levels were found This effect might have been due to decreased metabolism of T in the liver, which has also been suggested [17,19] The ability of bromocriptine to improve the T response to hCG in alcoholic rats supports the idea that prolactin has a role in the induction of hypogonadism by alcohol

Collectively, our data suggest that bromocriptine-induced suppression of plasma prolactin levels can exert a beneficial effect on the ethanol-induced hypogonadism shown in adult male rats

ACKNOWLEDGEMENTS

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