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Effects of bromocriptine on plasma testosterone and gonadotropin levels and testicular lipid fractions in adult rats

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Summary. Bromocriptine treatment of adult male rats resulted in a decrease in testicular testosterone (T) content and a reduction in plasma T levels. This was accompanied by increase in testicular total lipids and cholesterol and depletion of testicular phospholipids.

Administration of bromocriptine (CB-154), a dopaminergic agonist capable of suppressing prolactin (PRL) synthesis and release from pituitary acidophil cells, effectively decreases peripheral PRL levels in almost all species thus far investigated[2,3]. In rats, bromocriptine also influences the release of other gonadotropins[4] and a decreasing trend in peripheral testosterone (T) levels was observed in rats and mice injected with either CB-154 or with ergot alkaloids having similar biological activity[5]. Suppression of plasma T levels in these experiments could probably be explained by the CB-154-induced inhibition of PRL release[6] however, CB-154 appears to exert direct effects on Leydig cell function as well. Addition of CB-154 to the incubation media has been shown to inhibit T production by rat testicular cells in vitro[7], while lower concentrations of CB-154 stimulate basal T production by decapsulated mouse testes in vitro[8].

The mechanism(s) of bromocriptine action on testicular steroidogenesis remains to be elucidated; therefore, it was of interest to investigate the effects of bromocriptine injections on testicular lipid fractions, which include precursors for steroidogenesis.

Intact adult male Wistar rats (325 ± 10 g b.wt, 75 days of age) were purchased from Charles River Breeding Laboratories and maintained in a room with controlled illumination (14 h L: 10 h D) and temperature 24 ± 2 °C, with free access to food and water. Six rats received daily s.c. injections of CB-154 (Sandoz Pharmaceuticals, East Hanover, NJ; 1.0 mg in 0.1 ml sesame oil), while 6 rats received vehicle only. 24 h after the last injection, the animals were decapitated while under light ether anesthesia, and trunk blood was collected for plasma hormone assay. The testes were immediately removed, snap frozen and kept at −70 °C until further biochemical analysis. Epididymides, seminal vesicles and ventral prostates were removed and weighed. Plasma levels of LH, FSH, PRL and T and testicular levels of T were measured by radioimmunoassays described previously[8,9]. Testicular total lipids were extracted into Folch medium[10] and estimated colorimetrically according to the method of Frings et al.[11]. Phospholipids in the testis were estimated by the method described previously[12], by measuring the liberated inorganic phosphorus colorimetrically[13]. Free and esterified cholesterol concentrations were estimated colorimetrically after chromatographic separation of these 2 fractions[14].

In CB-154-treated animals, plasma PRL levels were suppressed to negligible values (table 1) in agreement with earlier observations[2,3,9]. Testicular weight seemed slightly reduced and plasma LH and FSH levels elevated, but these apparent differences were not statistically significant. The concentration of T in plasma and testes as well as the total testicular T content was significantly reduced in CB-154-injected rats (tables 1 and 2). The concentration and content of testicular phospholipids were decreased in the animals receiving CB-154 (table 2), while total lipid content of the testes and testicular cholesterol ester levels were increased with no alteration in free cholesterol. A significant reduction in plasma T levels and an apparent increase in plasma LH levels in the present study are reminiscent of the results of Boyns et al.[4] who reported a significant increase in plasma LH levels with simultaneous decrease in plasma T in bromocriptine-treated rats. The reduction in peripheral and testicular T levels probably resulted from PRL deficiency, since PRL increases the number of testicular LH receptors and potentiates LH action on the testis[6,15]. In this context, it is interesting to note the changes in testicular precursors of steroidogenesis after bromocriptine treatment. In hypophysectomized animals, combined deficiency of PRL and gonadotropins is accompanied by accumulation of lipids in seminiferous tubules[16,17] and alterations in testicular phopholipids[18]. In animals with isolated PRL deficiency in the present study, accumulation of cholesterol esters suggests that the activity of enzymes involved in the conversion of esterified cholesterol to T was inhibited. This could have been due to either direct effects of bromocriptine on the testes or to inhibition of PRL release with consequent reduction in the responsiveness of the testis to LH.

Table 1. Effects of bromocriptine (CB-154) injections on plasma prolactin (PRL), gonadotropin and testosterone (T) levels in mature male rats (means ± SD)

Hormone (ng/ml)	CB-154-treated (6)	Control (6)	Difference (%)
PRL	1.0 ± 0.2	49.0 ± 8.7	− 98*
T	2.98 ± 1.10	4.75 ± 0.97	− 37**
FSH	263 ± 84	231 ± 53	+ 14
LH	17 ± 12	12 ± 4	+ 40

*Significant at 0.1% level; **significant at 1% level.

Table 2. Effects of bromocriptine (CB-154) injections on testicular testosterone (T) levels and lipid fractions in mature rats (means ± SD)

Parameter	Concentration/g testis			Content/total testes		
	CB-154-injected	Control	Difference (%)	CB-154-injected	Control	Difference (%)
T (ng)	229 ± 55	292 ± 49	− 21.5*	710 ± 183	922 ± 38	− 23.0**
Total lipids (mg)	11.28 ± 1.60	9.2 ± 3.40	+ 23.6	34.5 ± 3.04	28.36 ± 8.22	+ 21.8*
Phospholipids (mg)	0.89 ± 0.09	1.27 ± 0.36	− 29.0**	2.70 ± 0.46	3.81 ± 1.27	− 29.0*
Cholesterol						
Esters (mg)	0.080 ± 0.019	0.052 ± 0.032	+ 54.0*	0.248 ± 0.061	0.163 ± 0.102	+ 51.7*
Free (mg)	0.812 ± 0.054	0.792 ± 0.041	+ 2.4	2.507 ± 0.248	2.544 ± 0.324	− 1.4

*Significant at 5% level; **significant at 1% level.

The decreased androgen levels in the testes are consistent with the decreased peripheral T titers and suggest that the reduction in plasma T in CB-154-treated animals was not due to increased metabolic clearance of T.

During active spermatogenesis and germ cell development, the testicular phospholipids have been reported to increase, with a decrease in neutral lipids[19-21]. Thus, the observed increase in total lipids content with decrease in the phospholipids fraction would suggest that CB-154 treatment can produce conditions unfavorable for normal spermatogenesis.

In summary, bromocriptine treatment of adult male rats resulted in a decrease in testicular T formation with a consequent reduction in plasma androgen levels. These effects were accompanied by changes in testicular total lipid, phospholipid and cholesterol content and seemed related to reduced testicular responsiveness to LH, since plasma LH levels were not suppressed.

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Influence of substance P and fragments on passive avoidance behavior

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Summary. N-terminal and C-terminal fragments of substance P (SP) have been shown to exert opposite effects on antinociception, grooming and fighting in mice. The present experiments explored whether these findings could be generalized to passive avoidance behavior. Substance P (SP-(1-11)) and the C-terminal fragment pyroglutamyl-SP-(7-11) attenuated passive avoidance behavior when picogram amounts were injected into the nucleus accumbens. In contrast, the N-terminal fragment SP-(1-7) had an opposite effect and facilitated passive avoidance behavior.

Brain peptides are precursor molecules of neuropeptides with different, opposite and selective CNS activities. For example, β-endorphin is a precursor of γ- and α-endorphin and their respective fragments (DTγE, DEγE, DTαE) which exert opposite effects on extinction of active and passive avoidance behavior[1]. Recently, Hall and Stewart[2] reported that the N-terminal SP-(1-7) and C-terminal pyroglutamyl-SP-(7-11) fragments of substance P exert opposite effects on several behavioral paradigms in mice. The present experiments were carried out to explore whether substance P and the N- and C-terminal fragments would also exhibit opposite effects in a learning paradigm.

Materials and methods. Animals. Male Wistar rats weighing 130-140 g were used. They were maintained under controlled conditions with a 12:12 light/dark cycle (light on between 07.00 h and 19.00 h), and received food and water ad libitum.

Implantation of cannulae into the brain. Rats were anesthetized with Hypnorm® and were secured in a stereotaxic instrument. Stainless steel cannulae (0.6 mm outer diame-