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The dopamine autoreceptor agonist B-HT 920 markedly stimulates sexual behavior in male rats

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Summary. B-HT 920, a selective agonist at dopamine (DA) autoreceptors, strongly increased the incidence of penile erections (PE) in male rats, an effect which was dose-related and antagonized by haloperidol. B-HT 920 at 100 and 200 μg/kg i.p. significantly altered the copulatory pattern of sexually active male rats, reducing the number of mounts and intromissions as well as the latency to the first ejaculation, a stimulant effect which was confirmed in sluggish males at a dose of 100 μg/kg.
Key words. B-HT 920; dopamine autoreceptors; penile erections; sexual behavior; haloperidol; rat.

There is now considerable evidence to indicate a fundamental role of dopaminergic pathways in the regulation of sexual behavior in male rats. All dopamine (DA) receptor stimulants as yet tested elicit penile erections (PE) in isolated males[1–5] and influence the whole copulatory pattern, facilitating performance of the test in normal rats and restoring normal mating behavior in a percentage of impotent animals[6–8]. Although extrapolation from animal data to man always requires due caution, there does seem, to be agreement between animal results and clinical findings[9,10]. B-HT 920 is reported to be a specific agonist at DA autoreceptors[11,12]. It was on account of this selectivity of action, lacking in all other dopaminergic drugs as yet tested, which act at both D_1 and D_2 receptors[13], that we decided to study the influence of B-HT 920 on the sexual activity of male rats in the presence and in the absence of females in estrus.

Materials and methods. Animals and treatment. Male Wistar rats (Morini Farm, S. Polo d'Enza, Reggio Emilia, Italy) 230–250 g initial weight, were used. Female rats used as mating stimulus were of the same strain and weight. The animals were housed in cages 50 × 25 × 20 cm, eight animals per cage, with water and food freely available, at 22 ± 2 °C with a relative humidity of 60% and 12-h light/dark cycles (light on from 06.00 to 18.00 h). Tests were performed between 09.00 and 12.00 h, including the copulatory tests, as previous tests had established that there is no significant difference between performance under natural and reversed light conditions. 15 min before tests the animals were placed in glass observation cages to accustom them to the new environment. When animals were pretreated with the antagonist haloperidol, the drug was injected 45 min before B-HT 920. The substances were dissolved in distilled water and injected i.p. at a constant volume of 2 ml/kg. Doses of drugs refer to the weight of the salt. Controls were given the same volume of vehicle.

Evaluation of penile erections (PE) in male rats in absence of females. Immediately after the i.p. injection of B-HT 920 animals, in groups of 4 or 5 rats each, were observed continuously for 1 h by experienced researchers not aware of the animals' treatment. Each occurrence of PE was recorded for each animal responding during the observation period and the percentage of animals responding was calculated as well as the mean number ± SEM of PE per animal responding. The data for PE were analyzed by Student's t-test, with the level of significance set at $p < 0.05$.

Evaluation of male copulatory behavior. Female rats used as mating stimulus were brought into estrus with a s.c. injection of 0.12 mg estradiol benzoate 48–72 h before use. Male copulatory behavior was evaluated as by Dewsbury[14] and the following were recorded: mount and intromission latencies (ML and IL) (time elapsed from the introduction of the female into the cage until the 1st mount and intromission, respectively), mount and intromission frequency (MF and IF) (number of mounts and intromission preceding ejaculation), ejaculation latency (EL) (interval from the 1st intromission to ejaculation), postejaculatory interval (PEi) (time from the 1st ejaculation to the new 1st intromission). Tests were discontinued when IL or PEi were > 15 min or EL was > 30 min. Of a large number of male rats at the start of the experiments 22 were considered sexually active, five impotent and five sluggish, the remainder with no fixed pattern of behavior were discarded. Sexually active males were those which performed completely at least the last five preliminary mating tests out of the six conducted at 3-day intervals. Impotent males were those which never responded in any of the six preliminary mating tests. Sluggish males were those which, although showing a tendency to perform the test, failed to reach ejaculation in at least the last three out of the six preliminary tests.

The degree of constancy of copulatory behavior in the 5th and 6th pretests was checked with Student's t-test for paired data, which was also used to compare the values obtained before (mean of 5th and 6th tests) and after administration of B-HT 920 (level of significance set at $p < 0.05$), which was i.p. injected 15 min before the start of the final test.

Drugs. The following drugs were used. B-HT 920 (2-amino-6-allyl-5,6,7,8-tetrahydro-4H-thiazolo-4,5-d-azepine 2 HCl; Boehringer Ingelheim, Ingelheim am Rhein), haloperidol (Serenase, Lusofarmaco, Milan).

Results. Behavioral effects induced by B-HT 920 in males in absence of females. Consistently with previous results[5] control rats displayed PE in the 1-h observation period though the episodes were rare and restricted to a small percentage of animals (fig.). I.p. injection of B-HT 920 increased the percentage of animals responding, as well the mean number of PE per animal responding. The figure shows the impressive activity of

the dopaminergic agonist as an inducer of PE, the number of occurrences of which were dose-related and significantly enhanced with respect to controls at doses from 10 to 1000 µg/kg. The maximal effect was obtained with 100 µg/kg and was higher than any previously obtained with dopaminergic stimulants[4,5]. In line with previous reports[11,12] B-HT 920 was found not to induce stereotyped behavior (SB). A clear dose-related antagonism of PE (significant both as regards the mean number of occurrences per animal responding and as regards the percentage of animals responding) was obtained after i.p. pretreatment with haloperidol at doses of 0.025 mg/kg and above (table 1).

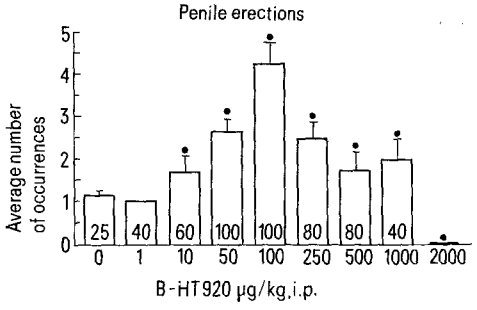
Effect of B-HT 920 pretreatment on copulatory performance of male rats. Table 2 shows the influence of B-HT 920 administered i.p. 15 min before the test, on the mating parameters in sexually active rats. Despite its sedative effect, at 100 and 200 µg/kg, B-HT 920 significantly diminished MF and IF as well as EL, effects interpretable as expressions of sexual stimulation. This stimulant activity was confirmed in the sluggish males which successfully performed the entire test after treatment with 100 µg/kg of B-HT 920. Of the parameters considered, ML, IL, MF and IF did not differ significantly from those of sexually active B-HT 920-treated rats, while LE and PEi were increased (to 518.75 ± 125.43 and 534.25 ± 75.32 sec respectively). No result was obtained however in the impotent rats, a failure which is probably to be attributed, to a certain extent at least, to the evident sedative effect of B-HT 920.

Discussion. The results obtained with B-HT 920 reported here once more support the role of DA in the regulation of sexual activity in male rats, suggesting moreover that the ability of a drug to evoke PE is predictive of its influence on mating behavior. The dopaminergic nature of PE was confirmed, in our experiments, by the clear dose-dependent antagonism of B-HT 920-induced PE exerted by haloperidol, which was significant even at 0.025 mg/kg and reached control values at 1 mg/kg. It would seem likely that the mechanism involved in the stimulation of PE by B-HT 920 is central rather than peripheral in that PE induced by other dopaminergic agents was not antagonized by pretreatment with domperidone, an inhibitor of extracerebral DA-receptors[4]. B-HT 920, considered to be a selective DA autoreceptor agonist[11,12], induced PE in rats at a large range of doses, with no sign of SB, while the dopaminergic agonists active both on pre- and postsynaptic receptors in general share an ability to elicit PE chiefly at low doses[4,15], doses which are reported to act selectively on DA autoreceptors[16,17], being progressively less effective on PE at the higher doses which elicit SB, with eventual total suppression of the normal occurrence of PE. It would seem, therefore, that the mechanism inducing PE must differ from that underlying SB, which apparently involves postsynaptic DA-receptor stimulation[13]; the impressive induction of PE by B-HT 920 would suggest the participation of DA autoreceptors in this case too. However, since B-HT 920 reduces DA synthesis and release and no PE stimulation was observed when DA transmission was impaired with other means, such as neuroleptics or 6-hydroxydopamine-induced lesions of brain DA-neurons[4], the precise mechanism remains unclear. Quite apart from the mechanism underlying PE-induction, however, the impressive sexual stimulant effect of B-HT 920 in male rats deserves consideration in view of the need for a better understanding of ejaculation disorders in man and their possible treatment.

Table 1. Effect of pretreatment with haloperidol on penile erections (PE) induced by B-HT 920 100 µg/kg in male rats

Pretreatment (mg/kg i.p.)	B-HT 920 (µg/kg i.p.)	Average number of PE per animal responding
–	–	1.17 ± 0.08 (25)
–	100	4.25 ± 0.49 (100)[a]
haloperidol 0.012	100	2.80 ± 0.58 (100)
haloperidol 0.025	100	2.00 ± 0.37 (60)[b]
haloperidol 0.25	100	1.00 ± 0 (50)[b]
haloperidol 0.5	100	1.20 ± 0.20 (40)[b]
haloperidol 1.0	100	1.00 ± 0 (20)[b]

B-HT 920 was injected i.p. immediately before the 1 h observation period. Haloperidol was administered 45 min before B-HT 920. Each value represents the mean ± SEM of PE per animal responding. In parentheses the percentage of animals responding, based on at least 10 rats per treatment group. [a] $p < 0.001$ with respect to controls. [b] $p < 0.05$ at least, with respect to B-HT 920-treated animals.



Influence of B-HT 920 on penile erections (PE) in male rats. B-HT 920 was injected i.p. immediately before the 1-h observation period. Each histogram represents the mean ± SEM of PE per animal responding. Percentage of animals responding is shown within the histogram. Values are based on at least 10 rats per treatment group. ● $p < 0.05$ at least, with respect to controls (Student's t-test).

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Table 2. Influence of B-HT 920 on the copulatory behavior of sexually active male rats

Experiment	Treatment (µg/kg i.p.)	ML (sec) (mean ± SEM)	IL (sec)	MF	IF	EL (sec)	PEi (sec)
1 (6)	Saline	4.83 ± 0.95	9.17 ± 2.24	5.58 ± 1.11	8.83 ± 0.63	273.33 ± 27.81	352.25 ± 5.02
	B-HT 920,50	5.67 ± 0.67	10.33 ± 1.52	4.00 ± 1.03	8.67 ± 0.33	296.67 ± 33.93	380.83 ± 17.77
2 (8)	Saline	5.43 ± 1.62	9.42 ± 1.56	6.07 ± 0.59	9.43 ± 0.74	247.14 ± 29.10	324.57 ± 19.68
	B-HT 920, 100	6.17 ± 1.45	26.17 ± 11.52	3.67 ± 0.42*	5.67 ± 0.80*	153.33 ± 22.50*	324.83 ± 63.54
3 (8)	Saline	6.17 ± 1.85	7.83 ± 1.72	6.58 ± 0.87	9.92 ± 0.62	260.17 ± 32.12	322.83 ± 12.44
	B-HT 920, 200	5.83 ± 1.40	8.67 ± 1.36	3.00 ± 1.05*	4.00 ± 0.86*	120.50 ± 30.58*	380.17 ± 20.36

ML, IL, EL: latency to the 1st mount, 1st intromission and ejaculation, respectively. MF, IF: mount and intromission frequency, respectively. PEi: post-ejaculatory interval. The saline values are the mean of 5th and 6th tests, the constancy of copulatory behavior from one test to the other having previously been ascertained ($p > 0.05$: Student's t-test for paired data). The saline values obtained for the various groups of randomly selected animals do not differ significantly from one another ($p > 0.05$: analysis of variance). In parentheses the number of rats.* A least $p < 0.05$ with respect to saline (Student's t-test for paired data).

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Proteolytic inactivation of human leukocyte elastase

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Summary. Human leukocyte elastase can be proteolytically inactivated by bovine pancreatic trypsin. Neither porcine pancreatic elastase nor bovine pancreatic chymotrypsin causes inactivation of leukocyte elastase, nor are trypsin, pancreatic elastase, or chymotrypsin themselves susceptible to proteolysis. The trypsin-catalyzed inactivation of leukocyte elastase can be slowed by inhibition of trypsin with benzamidine or by occupation of elastase's active site with elastatinal.
Key words. Elastase; inactivation, proteolytic.

Leukocyte elastase (EC 3.4.21.11) is thought to be the principle pathogenic agent involved in the development of pulmonary emphysema[1,2], a disease characterized by the progressive degradation of lung connective tissue, especially elastin[2,3]. Advances towards understanding the role played by this serine protease in emphysema have relied in large part on animal models. Emphysema can be mimicked in laboratory animals by intratracheal instillation of an elastolytic enzyme, typically porcine pancreatic elastase (PPE)[4]. Depending upon the dose, this single insult can result in a lesion that progressively worsens throughout the lifetime of the animal[4].

In attempts to potentiate the lesion by co-administration of elastase and another protease, we, and others, have found that bovine pancreatic trypsin (BPT) when administered with PPE to hamsters produces a significant enhancement of the resultant PPE-induced emphysema[5,6]. Surprisingly, we recently found that if trypsin is administered with human leukocyte elastase (HLE) the resultant lesion is significantly *diminished*[5]. Similarly, we observed that rates of elastinolysis were diminished by trypsin[7]. To explain the results regarding HLE we postulated two potential mechanisms[7]: a) the proteolytic inactivation of HLE by BPT or b) the generation of inhibitory peptides by BPT assisted elastinolysis. We now report results which demonstrate that HLE is in fact susceptible to proteolytic destruction by BPT.

Materials and methods. Human leukocyte elastase, prepared by the method of Viscarello et. al.[8], and porcine pancreatic elastase were obtained from Elastin Products, Pacific, MO. BPT, CT, and benzamidine were from Sigma Chemical Co. St. Louis, MO. Elastatinal was purchased from Vega Biochemicals, Tucson, Arizona.

Assays. HLE activity was measured with the chromophoric substrate MeOSuc-Ala-Ala-Pro-Val-pNA as previously described[8,9]. PPE[10], BPT[8], and CT[8], were also measured as previously described. Concentration of BPT active sites was determined with the titrant p-nitrophenyl-p'-guanidinobenzoate[11]. HLE concentration was determined from established kinetic constants for the hydrolysis of MeOSuc-Ala-Ala-Pro-Val-pNA[9].

Inactivation of HLE. The inactivation of HLE was followed by periodically assaying small aliquots (10–50 µl) of reaction solutions of HLE and BPT for residual activity against MeO-Suc-Ala-Ala-Pro-Val-pNA. Semi-log plots of residual HLE-activity vs time were linear for at least three half-lives.

Results. Susceptibility of HLE to proteolytic inactivation. Figure 1 contains results of an experiment in which HLE alone or in combination with equimolar concentrations (1µM) of either PPE, CT, of BPT was incubated and periodically assayed for HLE activity. It is apparent that while HLE is inactivated by BPT it is resistant to proteolysis by PPE or CT. The loss of

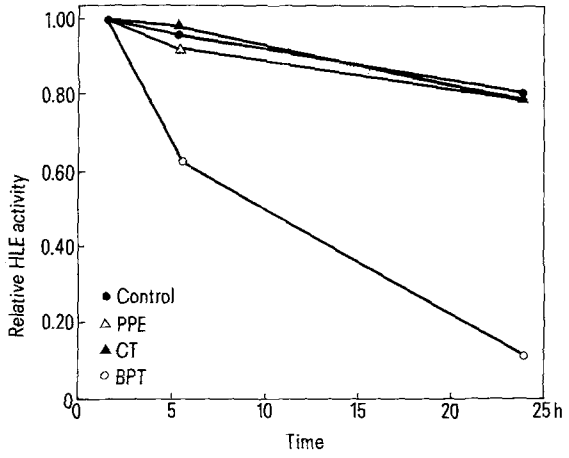

Figure 1. Effect of proteases on HLE activity. 1 µM HLE and an equimolar amount of another protease were incubated in 0.1 M Tris, 0.5 M NaCl, pH 8.0, 20 °C. Residual HLE activity is expressed relative to activity at time equal 1 min.