Psychopharmacology (1992) 107:327–330

Psychopharmacology

Effects of fenfluramine and para-chloroamphetamine on sexual behavior of male rats

Mark M. Foreman, James L. Hall, and Ronald L. Love

Lilly Research Laboratories, Eli Lilly and Company, Lilly Corporate Center, Indianapolis, IN 46285, USA

Received July 22, 1991 / Final version November 4, 1991

Abstract. The present studies have evaluated the effects of pharmacologically induced release serotonin on sexual responses of male rats during exposure to a sexually receptive female rat. Following acute administration of fenfluramine or para-chloroamphetamine (PCA), significant dose-related decreases in copulatory rate and copulatory efficiency, and increases in ejaculatory latency were observed. These effects were not observed when the animals were pretreated with LY53857, a 5–$HT_{1c/2}$ antagonist. These studies indicate that acute release of serotonin evoked by these releasing agents has inhibitory effects on sexual sexual drive, capacity to achieve erection and threshold for ejaculation, and these effects are mediated by either the 5–HT_{1c} or 5–HT_2 receptor.

Key words: Sexual behavior – Serotonin – Fenfluramine – Para-chloroamphetamine – LY53857 – 5–HT_{1c} receptor and 5–HT_2 receptor

Sexual response in the male rat can be both augmented or suppressed by serotonergic receptor stimulation depending upon the type of receptors affected. Copulatory behavior of male rats is inhibited by administration of the 5–$HT_{1c/2}$ agonist, DOI; non-selective agonists e.g. quipazine and 5–MeO–DMT or administration of 5–HT reuptake inhibitors but is stimulated by treatment with serotonergic neurotoxins; 5–HT_{1a} agonists or selective 5–HT antagonists (Hamburger-Bar et al. 1978; Larsson et al. 1978; Ahlenius et al. 1980, 1981, 1989; Baum and Starr 1980; Mendelson and Gorzalka 1985; Glaser et al. 1987; Menendez et al. 1988; Fernández-Guasti et al. 1989; Foreman et al. 1989). Since increasing postsynaptic serotonergic response by administration of reuptake inhibitors or 5–$HT_{1c/2}$ agonists suppresses sexual behavior and decreasing this response by administration of agonists for the 5–HT_{1a} autoreceptor or postsynaptic antagonists increases sexual behavior, these studies suggest an inhibitory role for serotonin in the control of sexual behavior in the male rat.

An alternative method of evaluating the role of 5–HT in various biological responses is to evoke the release of 5–HT by administering fenfluramine or para-chloroamphetamine (PCA). Effects on serotonin mediated behaviors noted following acute administration are related to an increased serotonin release evoked by these compounds whereas the effects observed after repeated administration at higher doses are related to a decreased serotonin release due to the neurotoxic effects of these compounds (Clineschmidt et al. 1978; Sanders-Bush and Steranka 1978; Fuller et al. 1988; Schwartz et al. 1989; Appel et al. 1989). Previously, PCA has been reported to suppress lordotic responses of estrogen and progesterone treated, ovariectomized female rats following acute treatment (Zemlan et al. 1977; Zemlan 1978; Yamanouchi et al. 1982) and to stimulate mating behavior of orchidectomized, male rats following repeated administration (Södersten et al. 1978). These experiments suggest that sexual response can be suppressed or augmented by drug treatments that increase or decrease, respectively, serotonin release. However, acute administration of pCA or fenfluramine have also been shown to induce erections and ejaculation in rats and rhesus monkeys (Rényi 1985, 1986; Berendsen and Broekkamp 1987; Szele et al. 1988; Berendsen et al. 1990). Thus, 5–HT may have varied effects on sexual response depending upon the receptor type, location of those receptors in the neural network regulating sexual response and the types of response model used.

The focus of the present studies was to evaluate the effects of acute administration of fenfluramine or PCA on performance parameters of male rat sexual responses including the ejaculatory latency, copulatory efficiency and copulatory rate.

Offprint requests to: M.M. Foreman

Materials and methods

Materials. Fenfluramine and DL-para-chloroamphetamine (PCA) were purchased from Sigma Chemical Co. (St. Louis, MO). LY53857 was synthesized at the Lilly Research Laboratories.

Behavioral testing procedure. All of the rats used in these studies were housed in a temperature controlled room in which the lights were off from 10:00 to 20:00. Male rats of the Sprague-Dawley strain and female rats of the Long-Evans strain purchased from Charles River Breeding Laboratories (North Wilmington, MA) were used in these studies. Each of the female rats was ovariectomized under ether anesthesia and allowed a 4 week postsurgical recovery period. The ovariectomized rats were used as sexual partners for the test males and were made sexually receptive by administering 400 μg estrone in propylene glycol SC 48 h prior to testing and 2.5 mg progesterone in propylene glycol SC 4 h prior to testing. Each male rat was individually housed beginning 4 weeks prior to testing. The sexual behavior of each rat was evaluated at 2 week intervals beginning at 6 months of age and ending at 12 months of age.

The mating tests were performed between 12:00 and 17:00 during the dark phase of the lighting cycle. The behavioral arenas used in these tests were plastic boxes measuring 61 cm × 61 cm at the base and 31 cm in height, which were illuminated with red lights. Each behavioral test was initiated with the introduction of a receptive female rat into the arena and was terminated either 30 min later or immediately following the first postejaculatory mount. The types of mating performance indices that were used included the ejaculatory latency (time interval between the first intromission and ejaculation), copulatory efficiency (number of intromissions achieved per total number of mounts) and copulatory rate (number of mounts per minute). The 145 animals used in these studies had baseline mean (±SEM) ejaculatory latencies, copulatory rates and copulatory efficiencies of 325.5±9.7s, 3.15±0.09 mounts per min and 0.53±0.04 intromissions per mount, respectively.

Unless otherwise indicated, all compounds used in these studies were administered 30 min prior to behavioral testing by sc injection. A vehicle consisting of 1 mM ascorbic acid and 1 mM acetic acid was used for all drug solutions. Prior to treatment with a drug solution, each male rat was required to have at least two consecutive vehicle tests with similar sexual performance. Following each drug testing, additional vehicle tests were performed to comfirm baseline activity levels. In an effort to eliminate behavioral responses that may be due to spontaneous changes in baseline mating performance, a criterion of reversibility of behavioral response with subsequent vehicle treatment was employed (Foreman and Hall 1987; Foreman et al. 1989). Thus, a valid behavioral response to a drug treatment was arbitrarily set as a response that either did not change from the prior control response or was reversed in the subsequent control test with vehicle. Since this resulted in the elimination of some animal responses, the sample sizes for each treatment group varied from 9 to 12 rats. The predrug and postdrug vehicle responses of the animals included in these experiments were not statistically different. Animals that did not achieve ejaculation within the 30 min test period were assigned an ejaculatory latency of 1800s for statistical comparison. A paired t analysis was used for matched comparisons of performance values obtained from each rat following vehicle treatment (2 weeks prior to the drug treatment) and following a drug treatment. The minimum level of significance used in these studies was $P<0.05$.

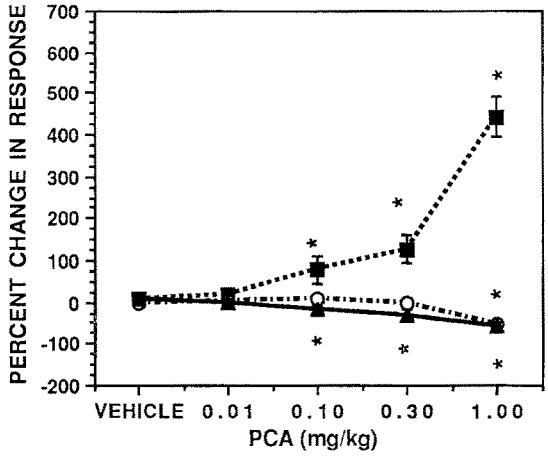

Fig. 2. Effects of para-chloroamphetamine on ejaculatory latency (--■--), copulatory rate (-▲-) and copulatory efficiency (--○--) of male rats. *Symbols* and *brackets* represent the mean and sem, respectively, and an *asterisk* denotes significant change from vehicle response. The minimum number of rats tested in each treatment was 12

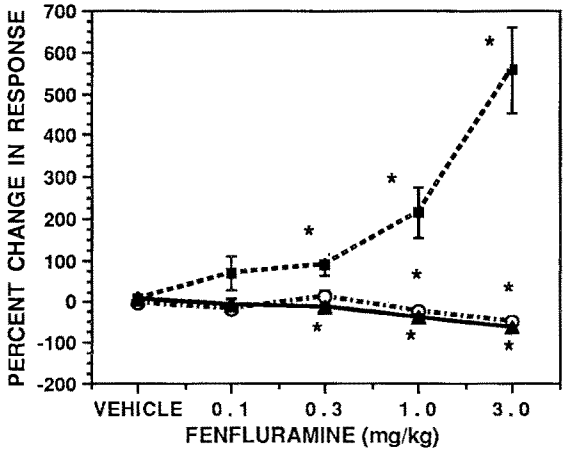

Fig. 1. Effects of fenfluramine on ejaculatory latency (--■--) copulatory rate (—▲—) and copulatory efficiency (--○--) of male rats. *Symbols* and *brackets* represent the mean and SEM, respectively, and an *asterisk* denotes significant change from vehicle response. The minimum number of rats tested in each treatment was 9

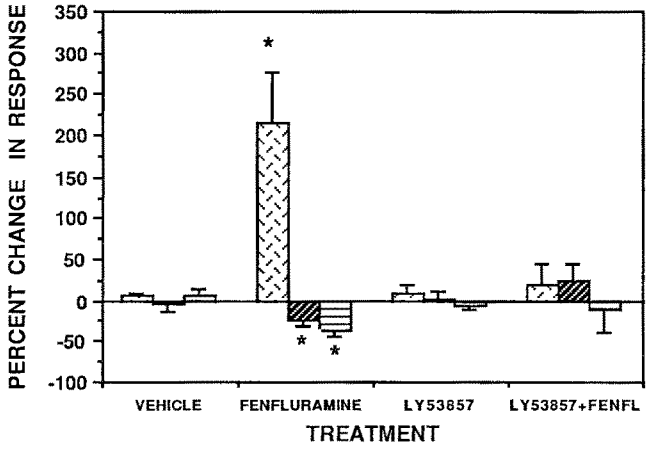

Fig. 3. Effects of pretreatment with LY53857 (0.1 mg/kg SC) on the changes in copulatory performance induced by fenfluramine (1.0 mg/kg SC). *Bars* and *brackets* represent the mean and SEM, respectively, and an *asterisk* denotes significant change from vehicle response. The minimum number of rats tested in each treatment was 9. (▨) Ejaculatory latency; (▨) copulatory rate; (▤) copulatory efficiency

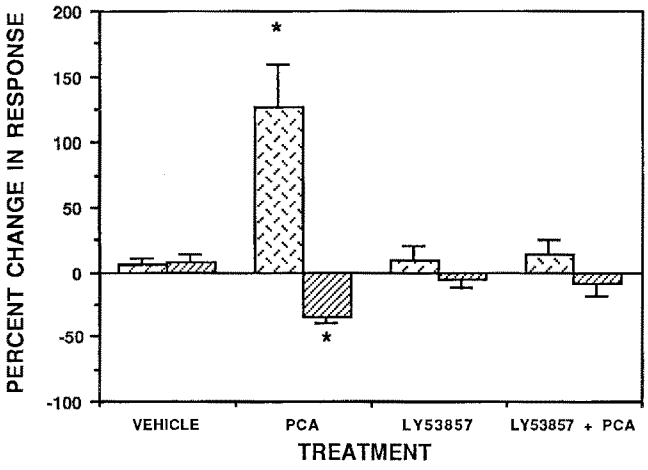

Fig. 4. Effects of pretreatment with LY53857 (0.1 mg/kg SC) on the changes in copulatory performance induced by para-chloroamphetamine (0.3 mg/kg SC). *Bars* and *brackets* represent the mean and SEM, respectively, and an *asterisk* denotes significant change from vehicle response. The minimum number of rats tested in each treatment was 12. (▨) Ejaculatory latency; (▨) copulatory rate

Results

Figures 1 and 2 contain summaries of the changes in ejaculatory latency (EL), copulatory efficiency (CE), and copulatory rate (CR) following administration of fenfluramine and para-chloroamphetamine (PCA), respectively. The minimum doses of fenfluramine and PCA required to significantly increase EL were 0.3 and 0.1 mg/kg, respectively. At the 1.0 and 3.0 mg/kg doses of fenfluramine, 21 and 59% of the rats, respectively, did not achieve ejaculation and at the 0.3 and 1.0 mg/kg doses of PCA, 14 and 80% of the rats, respectively, did not achieve ejaculation during the test period. The minimum doses of fenfluramine which significantly decreased CR and CE were 0.3 and 1.0 mg/kg, respectively. The minimum doses of PCA which significantly decreased CR and CE were 0.1 and 1.0 mg/kg, respectively.

Figures 3 and 4 contain summaries of the effects of pretreatment 60 minutes prior to testing with 0.1 mg/kg LY53857, a 5–$HT_{1c/2}$ receptor antagonist, on the changes in sexual responses following administration of vehicle, 1.0 mg/kg fenfluramine or 0.3 mg/kg PCA 30 min prior to testing. LY53857 had no significant effect on any of these response parameters following vehicle administration, but blocked the effects of fenfluramine and PCA.

Discussion

The present studies have demonstrated an inhibitory effect of fenfluramine and pCA administration of copulatory response in the male rat as measured by changes in the CR, CE and EL. The reductions in CR induced by fenfluramine or pCA administration are indicative of a suppressive action of serotonin on sexual drive. The reductions in CE and increased in EL that were observed following fenfluramine and pCA treatment are indicative of an inhibitory effect on erectile and ejaculatory reflex responses, respectively. All of these effects were blocked by pretreatment with LY53857, a 5–$HT_{1c/2}$ receptor antagonist implicating these receptors in the mediation of this response. In the present studies, treatment with 0.1 mg/kg LY53857 alone did not significantly alter the sexual responses of these animals. However, this dose of LY53857 did significantly lower EL in rats which had an average EL that was twice as long as the average EL in the present studies (Foreman et al. 1989).

Although the present studies can not define the site of action of these compounds, one possible site is the medial preoptic area (MPOA) of the ventral diencephalon. This proposal is supported by evidence from studies on the neurochemical changes following mating events and by evaluations of sexual response changes following infusions of 5–HT into the MPOA. Mas and coworkers (1987) have shown that 5–HIAA levels in the MPOA increase immediately following ejaculation when sexual behavior is suppressed. Verma and coworkers (1988) observed decreases in the frequency of pursuit and mounting and increases in mount latency following infusion of 5–HT into the medial preoptic area (MPOA). The infusion of 5–HT into the MPOA not only suppresses sexual drive parameters but also blocks intromission and ejaculatory responses, indicating that neuropharmacological intervention at the MPOA can lead to an alteration of spinal reflex activity. Collectively, these studies are suggestive of both physiology and pharmacologic roles for 5–HT receptors in the MPOA in the control of sexual behavior and response thresholds. In addition to the indirect effects on the spinal reflexes mediated through the alteration in sexual drive or MPOA activity, direct pharmacological effects on the spinal motor neuron response may also be possible. Direct intrathecal infusion of 5–HT has also been shown to suppress intromission and ejaculatory responses (Svensson and Hansen 1984; Mas et al. 1985). Thus, the effects of increased 5–HT release in either the rostral or caudal ends of the sexual response pathways can have inhibitory effects on sexual responses observed *in copulo*.

The observations of suppressed erectile and ejaculatory response following fenfluramine or pCA treatment in the present studies are in conflict with the previous observations of spontaneous erection and/or ejaculation induced by serotonin releasing agents in *ex copula* experiments (Rényi 1986; Berendsen and Broekkamp 1987). The effects of fenfluramine and pCA in the current studies occurred at doses that were 10–20 × lower than those used to elicit spontaneous erections and ejaculation in the rat. The discrepancy in the doses and effects may reflect different sensitivities of these animal models or different serotonergic receptors activated by the released 5–HT.

An alternative explanation for the differences in the effects of fenfluramine and pCA on sexual behavior and erectile/ejaculatory reflex response is that these effects may be mediated by serotonergic receptors in different areas of the sexual response pathways. Although MPOA and intrathecal administration of serotonergic agonists suppresses copulatory performance, increases in intracavernous pressure and penile nerve firing rate induced by serotonergic agonists have been observed before and

after spinal cord transection (Steers and de Groat 1989). Therefore, it can be assumed that some of the receptors involved with the erectogenic response are located in the lower spinal cord or periphery, whereas the 5–HT receptors involved in the behavioral suppression and descending inhibition of copulatory responses should be located in supraspinal areas of this response tree.

In summary, inhibitory effects of fenfluramine and pCA, two 5–HT releasing agents, were observed on sexual drive (copulatory rate) and on sexual reflex response (ejaculatory latency and copulatory efficiency) of the male rat. It was suggested that the postsynaptic receptors that mediate these effects are either 5–HT_{1c} or 5–HT_2, since pretreatment with a 5–$HT_{1c/2}$ receptor antagonist, LY53857, blocks these effects. These effects of fenfluramine in the male rat appear to be similar to the side-effects of fenfluramine in humans, which include the suppression of libido and the suppression of erectile response (Pinder et al. 1975).

References

Ahlenius S, Larsson K, Svensson L (1980) Further evidence for an inhibitory role of central 5–HT in male rat sexual behavior. Psychopharmacology 68:217–220

Ahlenius S, Larsson K, Svensson L, Hjorth S, Carlsson A, Lindberg P, Wikström H, Sanchez D, Arvidsson L-E, Hacksell U, Nilsson JLG (1981) Effects of a new type of 5–HT receptor agonist on male rat sexual behavior. Pharmacol Biochem Behav 15:785–792

Ahlenius S, Larsson K, Arvidsson L-E (1989) Effects of stereoselective 5–HT_{1a} agonists on male rat sexual behavior. Pharmacol Biochem Behav 33:691–695

Appel NM, Contrera JF, De Souza EB (1989) Fenfluramine selectively and differentially decreases the density of serotonergic nerve terminals in rat brain: evidence from immunocytochemical studies. J Pharmacol Exp Ther 249:928–943

Baum MJ, Starr MS (1980) Inhibition of sexual behavior by dopamine antagonist or serotonin agonist drugs in castrated male rats given estradiol of dihydrotestosterone. Pharmacol Biochem Behav 13:57–67

Berendsen HHG, Broekkamp CLE (1987) Drug-induced erections in rats: Indications of $serotonin_{1b}$ receptor mediation. Eur J Pharmacol 135:279–297

Berendsen HHG, Jenck F, Broekkamp CLE (1990) Involvement of 5–HT_{1c}-receptors in drug induced penile erections in rats. Psychopharmacology 101:57–61

Clineschmidt BV, Zacchei AG, Totaro JA, Pflueger AB, McGuffin JC, Wishousky TI (1978) Fenfluramine and brain serotonin. Ann NY Acad Sci 305:222–241

Fernández-Guasti A, Escalante A, Ågmo A (1989) Inhibitory action of various 5–HT_{1b} receptor agonists on rat masculine sexual behavior. Pharmacol Biochem Behav 34:811–816

Foreman MM, Hall JL (1987) Effects of D_2-dopaminergic receptor stimulation on male rat sexual behavior. J Neural Transm 68:153–170

Foreman MM, Hall JF, Love RL (1989) The role of the 5–HT_2 receptor in the regulation of sexual performance of male rats. Life Sci 45:1263–1270

Fuller RW, Snoddy HD, Robertson DW (1988) Mechanisms of effects of *d*-fenfluramine on brain serotonin metabolism in rats: uptake inhibition versus release Pharmacol Biochem Behav 30:715–721

Glaser T, Dompert WU, Schuurman T, Spencer DG, Traber J (1987) Differential pharmacology of the novel 5–HT_{1a} receptor ligands 8–OH–DPAT, BAY R 1531 and ipsapirone. In: Dourish CT, Ahlenius S, Hutson PH (eds) Brain 5–HT_{1A} receptors. Ellis Horwood New York, NY pp 106–119

Hamburger-Bar R, Rigter H, Dekker I (1978) Inhibition of serotonin reuptake differentially affects heterosexual behavior of male and female rats. Life Sci 22:1827–1836

Larsson K, Fuxe K, Everitt BJ, Holmgren M, Södersten P (1978) Sexual Behavior in male rats after intracerebral injection of 5,7-dihydroxytryptamine. Brain Res 141:293–303

Mas M, Zahradnik MA, Martino V, Davidson JM (1985) Stimulation of spinal serotonergic receptors facilitates seminal emission and suppresses penile erectile reflexes. Brain Res 342:128–134

Mas M, Rodriguez del Castillo A, Guerra M, Davidson JM, Battner E (1987) Neurochemical correlates of male sexual behavior. Physiol Behav 41:341–345

Mendelson SD, Gorzalka BB (1985) Serotonin antagonist pirenperone inhibits sexual behavior in the male rat: attenuation by quipazine. Pharmacol Biochem Behav 22:565–571

Menendez AE, Moran VP, Velasco PA, Marin B (1988) Modifications of the sexual activity in male rats following administration of antiserotonergic drugs. Behav Brain Res 30:251–258

Pinder RM, Brogden RN, Sawyer PR, Speight TM, Avery GS (1975) Fenfluramine: a review of its pharmacological, properties and therapeutic efficacy in obesity Drugs 10:241–323

Rényi L (1985) Ejaculations induced by *p*-chloroamphetamine in the rat. Neuropharmacology 24:697–704

Rényi L (1986) The effect of selective 5-hydroxtryptamine uptake inhibitors on 5-methoxy-N,N-dimethyltryptamine-induced ejaculation in the rat. Br J Pharmacol 87:639–648

Sanders-Bush E, Steranka LR (1978) Immediate and long-term effects of *p*-chloroamphetamine on brain amines Ann NY Acad Sci 305:208–221

Schwartz D, Hernandez L, Hoebel BG (1989) Fenfluramine administered systemically of locally increases extracellular serotonin in the lateral hypothalamus as measured by microdialysis. Brain Res 482:261–270

Södersten P, Berge OG, Hole K (1978) Effects of *p*-chloroamphetamine and 5,7-dihydroxytryptamine on the sexual behavior, of gonadectomized male and female rats Pharmacol Biochem Behav 9:499–508

Steers WD, de Groat WC (1989) Effects of *m*-chlorophenylpiperazine on penile and bladder function in rats Am J Physiol 257:R1441–1449

Svensson L, Hansen S (1984) Spinal monoaminergic modulation of masculine copulatory behavior in the rat Brain Res 302:315–321

Szele FG, Murphy DL, Garrick NA (1988) Effects of fenfluramine, *m*-chlorophenylpiperazine, and other serotonin agonists and antagonists on penile erections in nonhuman primates. Life Sci 17:1297–1304

Verma S, Chhina GS, Kumar VM, Singh B (1988) Inhibition of male sexual behavior by serotonin application in the medial preoptic area Physiol Behav 46:327–330

Yamanouchi K, Watanabe H, Okada R, Arai Y (1982) Forebrain lordosis inhibiting system and serotonin neuron in female rats: effect of *p*-chloroamphetamine Endocrinol J 29:469–474

Zemlan FP (1978) Influence of *p*-chloroamphetamine and *p*-chlorophenylalanine on female mating behavior Ann NY Acad Sci 305:621–626

Zemlan FP, Trulson ME, Howell R, Hoebel BG (1977) Influence of *p*-chloroamphetamine on female sexual reflexes and brain monoamine levels Brain Res 123:347–356