Pharmacology Biochemistry and Behavior, Vol. 54, No. 1, pp. 189–193, 1996

0091-3057/96 $15.00 + .00

SSDI 0091-3057(95)02115-9

Involvement of the 5-HT_{1A} Subtype Receptor in the Neonatal Organization of Agonistic Behaviour in the Rat

E. ALBONETTI,* M. I. GONZÁLEZ,† A. SIDDIQUI,‡ C. A. WILSON†[1] AND F. FARABOLLINI*

*Institute of Physiology, University of Siena, Italy
†Department of Obstetrics and Gynaecology, St. George's Hospital Medical School, London SW17 ORE, UK
‡Department of Physiology, The Aga Khan University, Pakistan

ALBONETTI, E., M. I. GONZÁLEZ, A. SIDDIQUI, C. A. WILSON AND F. FARABOLLINI. *Involvement of the 5-HT_{1A} subtype receptor in the neonatal organization of agonistic behaviour in the rat.* **54**(1) 189–193, 1996.—5-hydroxytryptamine (5-HT) interacts with testosterone (T) in the development of a number of neuronal systems controlling sexually dimorphic adult behaviours. In this report, we investigated this interaction on the organization of agonistic behaviour in males, females, androgenized females (250 μg/pup of T proprionate on the day of birth), and males castrated on the day of birth. We have shown previously that manipulating 5-HT_2 activity over the 2nd week of life modulates adult agonistic behaviour, depending on genetic sex and the presence of T. In this report, we investigated the effects seen in adulthood of a 5-HT_{1A} agonist [8-OH-DPAT, 0.25 mg/kg, intraperitoneally (IP)] and antagonist (WAY100135, 0.25 mg/kg, IP) given over days 8–16 postpartum. The test for agonistic behaviour was carried out in a neutral territory against a matched conspecific, and introductory, offensive and defensive activities were noted. Results show that neonatal administration of the 5-HT_{1A} antagonist WAY100135 increases introductory activity and defense in the presence of neonatal T, independent of genetic sex, because these effects were seen in sham-castrated males and androgenised females. Offence followed a similar pattern, in that it was increased by WAY100135, but only in males. In the case of defence, the effects of the antagonist were reinforced by the action of the agonist (8-OH-DPAT) in both males and females, indicating an inhibitory role of 5-HT_{1A} perinatal activity on defence in the presence of malelike levels of circulating T and a facilitatory role when levels of T are low or negligible. These findings indicate that 5-HT_{1A} activity is involved in the development of agonistic behaviour and the effects are influenced by T. The results also show that the offensive and defensive facets of agonistic activity are controlled differently.

Neonatal Serotonin 5-HT_{1A} Adult agonistic behavior Sex differences

SEROTONIN (5-hydroxytryptamine; 5-HT) plays a major role in the control of a wide variety of brain functions. In particular, it is involved in the control of aggression in adult rodents (2), nonhuman primates (13,15), and humans (9,16). Among the many 5-HT-receptor subtypes identified in the last few years, 5-HT_{1A}, 5-HT_{1B}, 5-HT_{1C} (2C), 5-HT_2 (2A), and 5-HT_3 have until now been reported to be involved in the determination of agonistic activities in the adult (2).

In addition to its effects in adulthood, 5-HT is also thought to play an organizational role immediately after birth. We have shown that the neonatal interaction between 5-HT and testosterone (T) determines sexual dimorphism of several putative neural systems controlling adult behaviour (20,21). In the rat, during the 2nd week of life there is a reduction in hypothalamic 5-HT and its metabolite in males (14,21), which could have physiological significance in removing the inhibitory influence of the serotonergic system on T in the neonatal period (11). In the previous investigations, we manipulated the 5-HT_2-receptor subsystem at the 2nd week of life and showed that neonatal 5-HT_2 activity modulates adult agonistic behavior, depending on the genetic sex, hormonal neural substrate, and behaviour considered (1).

In the present work, we focused on the 5-HT_{1A}-receptor subtype. A number of papers point to it as one of the more

[1] To whom requests for reprints should be addressed.

important 5-HT receptors for the control of either aggression or anxiety-sustained defensive responses to aversive stimulation in adulthood. 5-HT_{1A} effects, however, vary depending on the route of administration [i.e., (8,10)] and, in the case of central administration, the brain area investigated (8), sex (4), and behaviour recorded. On the whole, in adult rodents, systemic administration of 5-HT_{1A} agonists appears to have an inhibitory influence on agonistic behaviour (2,5,17,18) in various experimental paradigms.

In the present investigation, we explored in two different experiments the possible influence of 5-HT_{1A}-system manipulation during the 2nd week of life over agonistic behaviour in adulthood of male and female rats, respectively. In addition, possible perinatal interactions between the 5-HT_{1A} subsystem and T were investigated. For this purpose, normal males and females, neonatally androgenized females, and neonatally castrated males were given, during the 2nd week of life, either the 5-HT_{1A} agonist 8-OH-DPAT or antagonist WAY100135. Agonistic and other social behaviours were measured in adulthood, in encounters with an unfamiliar conspecific of the same sex in a neutral environment.

METHOD

Animals

Twelve litters born to Wistar rats bred at St. George's Hospital Medical School were randomized and culled so that each dam had six males and six females.

Experiment 1

On the day of birth, the males of all litters were randomly assigned to one of two different groups: sham-castrated males and castrated males. All animals were anaesthesized by placing them in a freezer at −20°C for 10 min. Surgery consisted of making a small nick midscrotum and externalizing the testes in both castrated and sham groups, removing the testes in the former, and replacing under the skin in the latter; then the cut was stitched together. Afterward, the animals were returned to the mother so that lactation followed its normal course. The females of the litters were not used in this experiment.

Experiment 2

The females were assigned to one of two groups on the day of birth: normal and androgenized females. The androgenized females were subcutaneously (SC) injected with 250 μg/pup testosterone propionate (TP; Sigma Chemical Co., Dorset, UK) in 0.05 ml corn oil. Normal females received corn oil only. The males of the litters were not used in this experiment.

After day 1, the treatments, housing and testing conditions were identical for both experiments, except that the normal females were smeared daily from day 60 after birth.

On days 8–16 after birth, a third of the sham-castrated and castrated male groups, as well as a third of the normal and androgenized female groups, were treated daily with 0.25 mg/kg of the 5-HT_{1A} agonist 8-hydroxy-2-(di-*n*-propylamino)-tetralin (8-OH-DPAT) (Research Biochemicals International, Natick, MA); another third received 0.25 mg/kg of the 5-HT_{1A} antagonist WAY100135 (WAY) (*N-tert*-butyl-3-(4-(2-methoxyphenyl)piperazin-1-yl)-2-phenylpropionamide dihydrochloride; Wyeth Research Centre, Taplow, Bucks, UK). Both drugs were injected SC in 1 ml/kg saline. The remaining third of each group received saline. Animals from both experiments were weaned on day 21 and housed as same-sex and same-treatment groups of five to six. They were kept throughout life in a reversed-light 12 L : 12 D cycle (lights off at 0700 h) and observed under red illumination. Temperature was maintained at 22°C; food and water were available ad lib (Breeding Diet no. RM3; Special Diet Services, Lillyco, Surrey, UK). In Experiment 2, normal females were checked daily for the phase of their oestrous cycle by taking vaginal smears. In androgenized females, the vagina remained unopened.

Social behaviour test. Behavioural testing started at day 90 after birth. Each of the animals was introduced into a clean observation cage (43 × 21 × 181 cm) at the same time as an unfamiliar conspecific. The opponents were Wistar rats matched for sex, age, and body weight and day of cycle (for normal females) with the experimental animals, and housed under the same social and environmental conditions. The opponents were made familiar with the testing conditions at the beginning of the experiment by placing them in the observation arena for 2 consecutive days for 10 min each day. The tests were carried out between 1100 and 1700 h under dim red light and lasted 20 min. Normal females were tested on proestrus. The following behavioural categories were recorded.

Introductory behaviour [INTR; frequency (f) i.e., number of acts/20 min), including attend, approach, investigation, and anogenital sniffing.

Offence (OF; f), including aggressive allogrooming, offensive lateral threat, upright offence, biting, and on-top behavior. Despite probable differences in functions and brain mechanisms responsible for proximate causation (12), high-intensity offensive parameters (biting, on-top) were grouped together with low-intensity offensive parameters, because the former only had a very low overall frequency both in females and in males.

Defence (DE; f), including defence sideways posture, upright defence, retreating, and on-the-back posture.

Statistics

Data from Experiments 1 and 2 were analysed separately; nonnormal distributions were subjected to logarithmic transformation. For either experiment, two-way analysis of variance (ANOVA) was carried out, with factor 1 being a condition with two levels: sham-castrated and castrated for Experiment 1, and normal and androgenized for Experiment 2. Factor 2 for both experiments was treatment (three levels: saline, WAY100135, and 8-OH-DPAT). Whenever appropriate, specific cells in the design were compared by planned comparisons (22).

RESULTS

Experiment 1: Males

Means and SE of the results and the significance of differences between the groups are shown in Table 1.

In the case of INTR, two-way ANOVA resulted in a significant effect for main factors condition [$F(1, 35) = 15.35$, $p = 0.006$] and treatment [$F(2, 35) = 3.02$, $p = 0.05$]. Independently of treatment, frequencies were significantly higher in sham than castrated males. Independently of condition, frequencies were higher in WAY-treated than 8-OH-DPAT-treated males. However, a closer inspection of the data shows that this latter effect was mainly due to the sham rather than to the castrated group.

For OF, a significant effect was found for treatment only [$F(2, 35) = 5.30$, $p = 0.009$]. Planned comparisons showed

TABLE 1

EXPERIMENT 1: SOCIAL ACTIVITY TEST ON MALES (MEANS ± SEM)

Treat	INTR f	OF f[1]	DE f[1]
Sham saline (n = 10)	43.8 ± 7.6	8.2 ± 3.8	13.8 ± 5.4
Sham WAY (n = 6)	64.6 ± 8.0	19.6 ± 7.0	21.2 ± 5.6*
Sham 8-OH-DPAT (n = 6)	40.0 ± 7.2	1.0 ± 0.5	1.6 ± 0.8*‡
Castrated saline (n = 5)	29.6 ± 5.4	5.6 ± 3.6	10.4 ± 7.8
Castrated WAY (n = 8)	30.6 ± 3.6	9.0 ± 2.8	18.0 ± 5.8
Castrated 8-OH-DPAT (n = 6)	22.2 ± 4.0	3.6 ± 2.0	35.6 ± 11.8†

[1]Data transformed logarithmically. INTR = frequency of introductory behaviours (attend, approach, investigation, anogenital sniffing); OF = frequency of offence (aggressive allogroming, offensive lateral threat, upright offence, on-top, and biting); DE = frequency of defensive behaviours (defence sideways posture, upright defence, retreating, and on-the-back).

*p = 0.06; †p < 0.01, vs. saline, same condition.

‡p < 0.01 vs. other treatment, same condition.

that independently of condition, offence was significantly more frequent in WAY-treated than either saline- or 8-OH-DPAT-treated subjects (p = 0.02 and p = 0.003, respectively). Once again, this effect was mainly due to the sham rather than castrated group.

As for DE, a significant interaction between condition and treatment was found [$F(2, 35) = 5.85$, p = 0.006]. Planned comparisons indicated that 8-OH-DPAT treatment selectively depressed defence in sham males, while raising it in castrated males. Defence frequencies were indeed significantly lower in 8-OH-DPAT-treated sham males than in the WAY-treated sham males (p = 0.005) and in the corresponding saline group, though with a marginal significance (p = 0.06). On the other hand, 8-OH-DPAT-treated castrated males were more defensive than the animals of the corresponding saline group (p = 0.01). Finally, WAY-treated sham-males tended to be more defensive than saline ones (p = 0.06).

Experiment 2: Females

Means and SE of the results and significance of differences between the groups are shown in Table 2.

In the INTR behaviour, two-way ANOVA gave a marginally significant result for the interaction between androgenization and treatment [$F(2, 59) = 5.24$, p = 0.08]. Planned comparisons showed that WAY decreased INTR behaviour compared to the saline group in normal females (p = 0.04), while it increased it in androgenized females (p = 0.01).

For frequency of OF, two-way ANOVA resulted in no significant results.

As for DE, a significant Androgenization × Treatment interaction [$F(2, 59) = 6.83$, p = 0.002] was found. Planned comparisons indicated that defensive behaviour was increased by WAY in the androgenized females with respect to saline (p = 0.01) and 8-OH-DPAT group (p = 0.01). In normal females, the 8-OH-DPAT group was significantly higher than the WAY group (p = 0.01) and also higher than the saline group, with a marginal significance (p = 0.06).

DISCUSSION

In both male and female rats, perinatal manipulation of 5-HT_{1A} activity modified INTR and agonistic behaviours in adulthood. In males, the effects of the 5-HT_{1A} antagonist, WAY, and 5-HT_{1A} agonist 8-OH-DPAT were markedly different, depending on the presence or absence of testosterone. WAY increased INTR activities, OF, and DE in sham-castrated males and 8-OH-DPAT selectively affected DE, but with opposite actions in neonatally castrated and sham-castrated animals. Neonatal blockade of 5-HT_{1A} activity there-

TABLE 2

EXPERIMENT 2: SOCIAL ACTIVITY TEST IN FEMALES (MEANS ± SEM)

Treat	INTR f	OF f	DE f
Females saline (n = 11)	45.3 ± 4.2	8.7 ± 2.9	15.6 ± 4.8
Females WAY (n = 11)	33.4 ± 2.6†	11.2 ± 5.9	5.6 ± 1.7
Females 8-OH-DPAT (n = 9)	37.3 ± 2.7	5.8 ± 1.8	30.0 ± 15.7*§
Androgenized saline (n = 10)	31.4 ± 2.2	20.2 ± 6.4	14.8 ± 4.9
Androgenized WAY (n = 12)	46.4 ± 5.9‡	16.6 ± 7.4	38.7 ± 8.2‡§
Androgenized 8-OH-DPAT (n = 12)	39.2 ± 4.5	15.0 ± 6.1	9.3 ± 4.4

INTR = frequency of introductory behaviours (attend, approach, investigation, anogenital sniffing); OF = frequency of offence (aggressive allogroming, offensive lateral threat, upright offence, on-top, and biting); DE = frequency of defensive behaviours (defence sideways posture, upright defence, retreating, and on-the-back).

*p = 0.06; †p < 0.05; ‡p < 0.01, vs. saline, same condition.

§p < 0.01 vs. other treatment, same condition.

fore appeared to facilitate OF in adulthood, supporting similar results obtained after neonatal depletion of 5-HT by PCPA treatment over the 2nd week of life in males (7). This effect was basically confined to sham-castrated animals, with the castrated group showing lower levels of activity than in the intact rats, although the difference only reached significance for INTR activity.

In the case of DE, the action of the agonist and antagonist was clearly dependent on the presence of androgen in both males and females. Thus, in the presence of T, in intact males 8-OH-DPAT reduced DE behaviour compared to the saline-treated groups, and the same tendency was seen in the androgenised females. The antagonist WAY had the opposite effect and increased DE in both normal males and androgenised females. In contrast, in the absence of T, as seen in normal females and neonatally castrated males, 8-OH-DPAT significantly increased DE. Taking these results together, it seems that irrespective of genetic sex, but in the presence of malelike levels of circulating T, perinatal 5-HT_{1A} activity has an inhibitory role on adult DE behaviour and a facilitatory role when levels of T are low or negligable.

A possible explanation may be that T in some way alters the relative activity of pre- and postsynaptic 5-HT_{1A} receptors. Thus, one can speculate that endogenous 5-HT stimulates DE behaviour via postsynaptic receptors. However, in the presence of T, presynaptic 5-HT_{1A} receptors dominate, and the autoregulation of endogenous 5-HT release is enhanced and so postsynaptic activity is reduced. It is well established that steroids can alter serotonergic receptor density both at the membrane and genomic level (3,19); of particular interest, T can increase the affinity of 5-HT_{1A} receptors for 8-OH-DPAT (6).

Testosterone also appeared to influence the action of the 5-HT_{1A}-antagonist WAY on INTR behaviour, because this agent increased parameters of INTR activity in androgenised females, whereas in the absence of T (i.e., in normal females) it reduced activity. In contrast to its robust effect on DE, 8-OH-DPAT had little effect on INTR behaviour. Despite this, perhaps it can still be suggested that 5-HT_{1A} activity is inhibitory to INTR behaviour in the presence of T; that is, it reduced motivation to explore an unfamiliar conspecific, whereas a 5-HT_{1A} system may enhance this motivation in the absence of T.

Tentatively, the results on DE and INTR behaviour might suggest that the organizational activity of 5-HT_{1A} is mainly inhibitory in the presence of malelike levels of T. This applies to OF, too, although only in males, and so the relative weight of genetic sex and neonatal T in influencing 5-HT_{1A} perinatal activity may vary according to the behaviour considered. This is not surprising, given the variety of different neuronal mechanisms that control different behavioral systems [e.g., (12)].

Our results appear to differ from those obtained after neonatal manipulation of the 5-HT_2 receptors. The 5-HT_2 agonist DOI was found to increase DE independently of genetic sex, but only in the presence of malelike levels of circulating T; the 5-HT_2 antagonist ritanserin increased OF independently of sex and T levels, and raised DE only in normal males. The comparison between 5-HT_{1A} and 5-HT_2 results reveals neither common nor complementary patterns. This supports the general view that the two receptor subsystems—although both are involved in the organizational and activational control of social, and in particular, agonistic behaviours—mediate different and not necessarily related effects.

ACKNOWLEDGEMENTS

The authors are grateful for support for A. Siddiqui, on a travelling fellowship from the Aga Khan University, Pakistan, and for the generous gift of WAY100135 from Wyeth Research Centre, Taplow, Bucks.

REFERENCES

1. Albonetti, M. E.; Gonzalez, M. I.; Wilson, C. A.; Farabollini, F. Effects of neonatal treatment with DOI and ritanserin on agonistic behavior in adult male and female rats. Aggress. Behav. 20: 235–242; 1994.
2. Bell, R; Hobson, H. 5-HT_{1A} receptor influences on rodent social and agonistic behaviour: A review and empirical study. Neurosci. Biobehav. Rev. 18:325–338; 1994.
3. Biegon, A.; McEwen, B. S. Modulation by estradiol of serotonin receptors in brain. J. Neurosci. 2:199–205; 1982.
4. Blanchard, D. C.; Shepherd, J. K.; Carobrez, A. P.; Blanchard, R. J. Sex effects in defensive behavior: Baseline differences and drug interactions. Neurosci. Biobehav. Rev. 15:461–468; 1991.
5. Blanchard, R. J.; Yudko, E. B.; Rodgers, R. J.; Blanchard, D. C. Defense system psychopharmacology: An ethological approach to the pharmacology of fear and anxiety. Behav. Brain Res. 58:155–165; 1993.
6. Bonson, K. R.; Johnson, R. G.; Fiorella, D.; Rabin, R. A.; Winter, J. C. Serotonergic control of androgen-induced dominance. Pharmacol. Biochem. Behav. 49:313–322; 1994.
7. Farabollini, F.; Hole, D. R.; Wilson, C. A. Behavioral effects in adulthood of serotonin depletion by p-chlorophenylalanine given neonatally to male rats. Int. J. Neurosci. 41:187–199; 1988.
8. Graeff, F. G.; Silveira, M. C. L.; Nogueira, R. L.; Audi, E. A.; Oliveira, R. M. O. Role of the amygdala and periaqueductal gray in anxiety and panic. Behav. Brain Res. 58:123–131; 1993.
9. Hellings, J. A.; Warnock, J. K. Self-injurious behavior and serotonin in Prader-Willi syndrome. Psychopharmacol. Bull. 30:245–250; 1994.
10. Jenck, F.; Broekkamp, C. L. E.; Van Delf, A. M. L. 5-HT_{1C} receptors in the serotonergic control of periaqueductal gray induced aversion in rats. Psychopharmacology 100:372–376; 1990.
11. Johnson, H. M.; Payne, A. P.; Gilmore, D. P.; Wilson, C. A. Neonatal serotonin reduction alters the adult feminine sexual behaviour of golden hamsters. Pharmacol. Biochem. Behav. 35: 571–575; 1990.
12. Kruk, M. R. Ethology and pharmacology of hypothalamic aggression in the rat. Neurosci. Biobehav. Rev. 15:527–538; 1991.
13. Kyes, R. C.; Botchin, M. B.; Kaplan, J. R.; Manuck, S. B.; Mann, J. J. Aggression and brain serotonergic responsivity: Response to slides in male macaques. Physiol. Behav. 57:205–208; 1995.
14. Ladosky, W.; Gaziri, L. C. J. Brain serotonin and sexual differentiation of the nervous system. Neuroendocrinology 6:168–174; 1970.
15. Mehlman, P. T.; Higley, J. D.; Faucher, I.; Lilly, A. A.; Taub, D. M.; Vickers, J.; Suomi, S. J.; Linnoila, M. Low CSF 5-HIAA concentrations and severe aggression and impaired impulse control in nonhuman primates. Am. J. Psychiatry 151:1485–1491; 1994.
16. Rao, M. L.; Braunig, P.; Papassotiropoulos, A. Autoaggressive behavior is closely related to serotonin availability in schizoaffective disorder. Pharmacopsychiatry 27:202–206; 1994.
17. Sanchez, C. The role of serotonergic and adrenergic mechanisms in inhibition of isolation-induced aggression in male mice. Psychopharmacology 101:S50; 1990.

18. Sulcova, A.; Krsiak, M. Buspirone reduces aggressive behaviour in mice. Act. Nerv. Sup. (Pragua) 28:314–316; 1986.
19. Sumner, B. E. H.; Fink, G. Effects of acute estradiol on 5-hydroxytryptamine and dopamine receptor subtype mRNA expression in female rat brain. Mol. Cell. Neurosci. 4:83–92; 1993.
20. Wilson, C. A.; Gonzalez, I.; Farabollini, F. Behavioural effects in adulthood of neonatal manipulation of brain serotonin levels in normal and androgenized females. Pharmacol. Biochem. Behav. 41:91–98; 1992.
21. Wilson, C. A.; Pearson, J. R.; Hunter, A. J.; Tuohy, P. A.; Payne, A. P. The effect of neonatal manipulation of hypothalamic serotonin levels on sexual activity in the adult rat. Pharmacol. Biochem. Behav. 24:1175–1183; 1986.
22. Woodward, J. A.; Bonett, D. G.; Brecht, M. L. An introduction to linear statistical models for experimental design. New York: Harcourt Brace Jovanovich; 1986.