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Studies on the reproductive effects of chronic treatment with agomelatine in the rat

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ABSTRACT

Agomelatine is an antidepressant with a novel mechanism of action. It is a melatonergic agonist for MT_1 and MT_2 receptors and a serotonin (5-HT2C) receptor antagonist. Agomelatine has been suggested not to have adverse effects on sexual functions. However, the effects of chronic agomelatine administration on reproductive functions have not been sufficiently studied in animal models. We mainly aimed to explore the effects of agomelatine on reproductive functions in the male and female rats. For the experimental studies, Sprague Dawley rats were used. The animals started to receive daily oral agomelatine (10 mg/kg) on post-natal day 21. Agomelatine advanced vaginal opening in the female rats whereas it delayed puberty onset in the male rats. Agomelatine treatment significantly decreased intromission frequencies, which indicates a facilitator role of this antidepressant on male sexual behavior. In the forced swimming test (FST) used for assessing antidepressant efficacy, agomelatine induced a significant decrease in duration of immobility, and an increase in the swimming time, respectively, which confirms the antidepressant-like activity of agomelatine. The present findings suggest that agomelatine shows a strong antidepressant effect in the male rats without any adverse influences on sexual behavior, and its effects on pubertal maturation seem to show sex-dependent differences.

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1. Introduction

Antidepressants have been suggested to be divided into high risk (selective serotonin reuptake inhibitors, serotonin-norepinephrine reuptake inhibitors, tricyclic antidepressants, and monoamine oxidase inhibitors) and low risk (agomelatine, bupropion, moclobemide and reboxetine) categories with regard to propensity for antidepressant-induced sexual dysfunction (Keks et al., 2014). Dopamine agonists were reported to facilitate sexual behavior, and 5-HT is generally inhibitory. Norepinephrine agonists have dose-dependent effects, with low doses facilitating and high doses inhibiting sexual behavior (Hull and Dominguez, 2007). Most of the pharmacological agents which are used in the treatment of depression are known to disrupt normal sexual function

(Ferguson, 2001), which affects the patient's quality of life and represents the main side effect that leads to the discontinuation of treatment (Schweitzer et al., 2009). Agomelatine has been reported not to cause sexual dysfunction requiring treatment (Demyttenaere, 2011). All dose regimens of agomelatine have been reported to be well tolerated and have no unexpected adverse event (Kennedy et al., 2014). Understanding that there is a strong interaction between circadian rhythm desynchronization and mood disorders (Wirz-Justice, 2006) is another reason, which has led to the development of agomelatine. Agomelatine is a melatonergic agonist for MT_1 and MT_2 receptors and a serotonin (5-HT)$_{2C}$ receptor antagonist (de Bodinat et al., 2010). This compound was initially investigated as a chronobiotic; however, with the discovery of the 5-HT$_{2C}$ antagonist activity the emphasis shifted to its anxiolytic and antidepressant effects (Arendt and Rajaratnam, 2008). The antidepressant-like activity of agomelatine has been suggested to depend on a combination of its melatonin agonist and 5-HT$_{2C}$ antagonist properties (Papp et al., 2003; Bourin et al.,

2004). The antidepressant effect of agomelatine has been shown in several rodent models by using well established tests such as forced swimming test (FST) (Bourin et al., 2004) and chronic mild stress (Papp et al., 2003). Like other antidepressants, effects of agomelatine were principally apparent after chronic treatment. Therefore, in the present experiment, agomelatine administration was continued until 120 days whereas in the studies searching the antidepressant effect of agomelatine lasted only a few weeks. Since the use of melatonergic drugs in children and adolescents has been a matter of concern (Hardeland, 2009), one of the main objectives of this study was to evaluate the antidepressant effect of long-term agomelatine treatment by using the FST in male rats.

There are a few studies on sexual side-effects of agomelatine in the treatment of depression in humans. Agomelatine is suggested to demonstrate favorable sexual acceptability in depressed patients and healthy volunteers (Montejo et al., 2011) and therefore to be a good option for the treatment of depression because it would not have adverse effects on sexual function. Abnormal sperm parameters have been found to be associated with treatment with the SSRI citalopram but no similar association with agomelatine (Elnazer and Baldwin, 2014). The effects of agomelatine on sexual response have not been studied sufficiently in animal models although melatonin was previously shown to stimulate male sexual behavior in rats (Drago et al., 1999; Drago and Busa', 2000). Therefore, the main objective of the present experiment was to investigate the effects of agomelatine on sexual response in male rats. The effects of melatonin on pubertal maturation in male and female rats were also investigated as melatonin is generally suggested to have an inhibitory effect on onset of puberty.

2. Materials and methods

2.1. Animals and drug administration

Prepubertal Sprague-Dawley male and female rats, aged 21 days and weighing 40 ± 2 g, were obtained from the University of Firat Experimental Research Unit (Elazig, Turkey). The experimental protocols were approved by Firat University Ethical Committee, and the rats were treated in accordance with the national and international laws and policies on the care and use of laboratory animals. The animals were weaned at day 21 postpartum and were housed under a reversed light/darkness schedule (12 h light: 12 h darkness from 0700 h), at constant temperature $(21 \pm 1 °C)$ and humidity $(55 \pm 5\%)$ with free access to pelleted food and tap water. Tablet form of agomelatine was used to mimic the administration way in humans. Valdoxan tablets containing 25 mg agomelatine (Servier Industries, Wicklow Arklow/Ireland) were obtained from the local pharmacy. The required amount of tablets were crushed every day and suspended in saline. They were freshly prepared on the experiment day. Agomelatine (10 mg/kg) was orally given by gastric gavage daily at 9:00 h. Male and female rats received agomelatine from postnatal day (pnd) 21 (weaning day) to pnd 120 and 90, respectively. The control rats received a daily oral injection with saline in a volume of 1 ml/kg. Along treatment, the animals were monitored daily food and water intake and body weight gain. Preputial separation and vaginal opening (VO), morphological signs of the puberty in male and female rats, respectively, were evaluated from pnd 26 to puberty. In the female rats, sexual cycle was detected by daily vaginal cytology after the occurence of vaginal canalization. The experiment ended after pnd 90. On the afternoon of the first diestrus following 90 days, the rats were sacrificed. The male rats were maintained until pnd 120 for analyzing sexual behavior and antidepressant eficiacy.

2.2. Sexual behavior test

All sexual behavioral tests were performed during the dark phase between 13:00 h and 16:00 h in a room with night vision camera system. An IP day/night camera system was settled in the room where sexual behavior parameters were determined. The camera has a 1/3 type progressive scan sensor and infrared cut filters. It has a higher resolution (1920×1080) and respective frame rate (25 fbs). It has a light sensitivity of 0.05 lx/F1.2 for color and 0.005 lx/F.1 for white/black images. The day/night camera automatically switches from color to black and white mode depending upon avaliable light. It is dual codec and has three different compression standarts: JPEG, MPEG-4, MJPEG and H.264. The sexual performance of the rats was studied in two consecutive tests, the first pnd 90 or 91, the second pnd 105 or 106. Each rat was placed in a rectangular observation cage $(40 \times 50 \times 65)$ made from plexiglas. The rat was allowed to habituate for 15 min and then a receptive female was placed in the arena. The female rats bilaterally overiectomized three weeks before sexual behavior test were made sexually receptive by subcutaneous injection of estradiol benzoate (10 μg/rat) and progesterone (500 μg/rat) dissolved in 0.2 ml of sesame oil 48 and 6 h before the test session, respectively. Estradiol benzoate and progesterone were purchased from Sigma Chemical Co. (St. Louis, MO). Sexual behavior was recorded by the video-tape recording unit as described above. Each test lasted 30 min. Rats that did not ejaculate within 30 min were excluded from analysis. Video tape-recordings were later replayed and analysed in slow motion. The following parameters for the first two ejaculation series were measured: (ML) mount latency (time between the introduction of the female into the cage and the first mount); (IL) intromission latency (time between the introduction of the female into the cage and the first intromission); (EL) ejaculation latency (time between the first intromission and ejaculation); (EF) ejaculation frequency (number of ejaculations in each copulatory series); (PEI) postejaculatory interval (interval between each ejaculation and the next copulatory act); (MF) mount frequency (number of mounts prior to the first ejaculation); (IF) intromission frequency (number of intromissions prior to the first ejaculation); (copulatory effciency) intromission frequency/ intromission + mount frequency.

2.3. Forced swimming test

The FST (Porsolt et al., 1977) is the most widely used laboratory test for assessing the potential clinical antidepressant activity of drugs. The modified rat FST (Detke et al., 1995) was conducted. Chronic agomelatine-treated and vehicle-treated control rats were subjected to the FST. Tests were carried out two times, the first pnd 92 and the second pnd 107 on the day following each sexual behavior test. For pretest session, rats were individually placed for 15 min into plexyglass cylinders (60 cm height, 25 cm diameter) containing 39 cm of water at $25 \pm 1 °C$, so that the rat could not reach the floor without diving. After a pretest session of 15 min, the rat was removed, dried with a towel and returned to its cage. The cylinders were emptied and cleaned between rats. Following 24 h after their first exposures, the FSTs were performed. Each test was conducted for 5 min and behaviors were recorded by a videocamera placed above the cylinder for subsequent analysis. The observer was blind to the experimental conditions being scored. Immobility (floating), swimming and climbing were measured (Armario et al., 1988). When rodents are forced to swim in an inescapable situation, they typically display an immobile posture. When the animals become immobile, that is, floating in an upright position and making only small movements to keep their heads above water, the total duration of immobility was scored. Immobility reflects a state of "behavioral despair". Climbing behavior

consisted of struggling movements to get out the cylinder, with its forepaws above the surface of the water. Swimming behavior was defined as active swimming motions (usually horizontal) throughout the swim cylinder.

2.4. Reproductive organs collection

All animals were killed when the experiments ended after pnd 90 for female and pnd 120 for male rats. Blood was collected, and all reproductive tissues were dissected out and weighed.

2.5. Epididymal sperm analyses

All sperm analyses were made using the methods reported in the study of Turk et al. (2008). The sperm concentration in the right cauda epididymal tissue was determined with a haemocytometer. Freshly isolated left cauda epididymal tissue was used for the analysis of sperm motility. The percentage of sperm motility was evaluated using a light microscope with a heated stage.

2.6. Hormone measurements

Serum samples were stored at $-20\,°C$ until the assays were performed. Serum luteinizing hormone (LH) and follicle-stimulating hormone (FSH) were analyzed briefly as follows. Immunoplates (96-well) (Nunc, Roskilde, Denmark) were coated with rat LH or FSH. Serum samples or standards were pre-incubated with primary antibodies and were then transferred into coated plates for competition with antigens on the solid phase. Plates were washed and the secondary antibody conjugated to streptavidin peroxidase was added into each well and color was developed using tetramethylbenzidine as the substrate. Plates were read at an absorbance of 450 nm using a plate reader (Biotek Synergy HT, Winooski, VT, USA). Rat LH, rat FSH, and primary antibodies (rabbit anti-rat LH and rabbit anti-rat FSH) were obtained from Dr. AF Parlow (National Institute of Diabetes and Digestive and Kidney Diseases National Hormone and Peptide Program, National Institutes of Health, USA). Secondary antibodies (goat anti-rabbit IgG) conjugated to streptavidin peroxidase were purchased from Sigma-Aldrich. Sensitivity of the assays was 1 ng/ml for LH and 2 ng/ml for FSH. Inter- and intra-assay coefficient of variation values were below 8% for both LH and FSH.

2.7. Statistical analysis

Hormonal determinations were carried out in duplicate, with a minimal total number of 10 samples per group and values are expressed as mean $\pm$ S.E.M. Results were analyzed using Student's t test. For all analyses, $P < 0.05$ was considered statistically significant.

3. Results

3.1. Effects of agomelatine on sexual behavior in male rats

As seen in Tables 1 and 2, the average numbers of ejaculation were similar in the agomelatine-treated and control groups, being 2.33 ± 0.87 and 2.44 ± 0.53 in the first test session and 3.00 ± 0.33 and 2.5 ± 0.19 in the second test session, respectively. Agomelatine treatment significantly decreased intromission frequencies ($P < 0.05$, Table 1) in the first copulatory series in the first and second test sessions conducted on pnd 90 and 105, respectively. Total intromission frequency for 30-min test was also significantly decreased in the agomelatine-treated group in the first test session. Ejaculation latencies did not show significant changes

Table 1

Effects of chronic administration of agomelatine on sexual behavior in male Sprague-Dawley rats – 1st test pnd 90.

Parameter	Control	Agomelatine	P-value
1st copulation series			
ML (s)	85.0 ± 46.41	33.38 ± 12.3	0.300
IL (s)	24.75 ± 9.90	44.0 ± 15.88	0.321
MF (#)	8.13 ± 2.19	15.0 ± 3.38	0.110
IF (#)	27.13 ± 2.55	18.75 ± 1.91[a]	0.020
III (s)	9.36 ± 1.21	14.40 ± 2.39	0.081
EL (s)	450.57 ± 75.01	527.38 ± 89.07	0.528
PEI (s)	317.75 ± 29.84	403.13 ± 32.84	0.075
CE (%)	0.79 ± 0.04	0.60 ± 0.06[a]	0.016
2nd copulation series			
ML (s)	351.0 ± 45.39	346.43 ± 63.23	0.953
IL (s)	363.0 ± 18.9	412.25 ± 34.71	0.233
MF (#)	4.63 ± 1.13	6.86 ± 1.53	0.255
IF (#)	10.50 ± 1.74	11.13 ± 1.8	0.806
EL (s)	249.13 ± 48.97	223.5 ± 44.02	0.714
PEI (s)	453.83 ± 24.48	411.8 ± 16.9	0.209
EF (#)	2.44 ± 0.53	2.33 ± 0.87	0.747
MFT (#)	18.12 ± 3.02	24.38 ± 4.21	0.248
IFT (#)	44.75 ± 2.22	35.5 ± 3.09[a]	0.029
TCE (%)	0.72 ± 0.04	0.061 ± 0.04	0.073

Data is represented as mean ± S.E.M., ML=mount latency, IL=intromission latency, MF=mount frequency, IF=intromission frequency, III=inter-intromission interval for the first copulatory series, EL=ejaculation latency, PEI=post-ejaculatory-interval, CE=copulatory efficiency, EF=ejaculation frequency, MFT=total mount frequency preceding for 30-min test, IFT=total intromission frequency for 30-min test, TCE=total copulatory efficiency for 30-min test.

[a] $P < 0.05$ significantly different than control (Student' t test).

Table 2

Effects of chronic administration of agomelatine on sexual behavior in male Sprague-Dawley rats – 2nd test pnd 105.

Parameter	Control	Agomelatine	P-value
1st copulation series			
ML (s)	49.13 ± 20.54	69.0 ± 41.0	0.671
IL (s)	38.13 ± 17.38	27.25 ± 15.89	0.651
MF (#)	19.63 ± 6.0	7.38 ± 1.38	0.067
IF (#)	23.75 ± 2.09	14.88 ± 2.77[a]	0.023
III (s)	13.44 ± 1.25	13.57 ± 2.23	0.961
EL (s)	628.0 ± 67.3	381.25 ± 86.79[a]	0.041
PEI (s)	385.0 ± 31.65	359.0 ± 21.44	0.498
CE (%)	0.60 ± 0.06	0.66 ± 0.05	0.426
2nd copulation series			
ML (s)	356.63 ± 58.40	321.75 ± 87.45	0.745
IL (s)	390.88 ± 32.44	380.0 ± 25.6	0.796
MF (#)	10.88 ± 2.67	6.37 ± 2.49	0.238
IF (#)	8.38 ± 0.71	8.63 ± 0.8	0.818
EL (s)	236.0 ± 40.90	245.88 ± 54.32	0.889
PEI (s)	421.25 ± 15.94	449.33 ± 52.13	0.683
EF (#)	2.5 ± 0.19	3.0 ± 0.33	0.207
MFT (#)	33.13 ± 7.91	18.88 ± 3.20	0.117
IFT (#)	35.75 ± 2.26	32.25 ± 2.9	0.357
TCE (%)	0.55 ± 0.06	0.64 ± 0.05	0.326

Data is represented as mean ± S.E.M., ML=mount latency, IL=intromission latency, MF=mount frequency, IF=intromission frequency, III=inter-intromission interval for the first copulatory series, EL=ejaculation latency, PEI=post-ejaculatory-interval, CE=copulatory efficiency, EF=ejaculation frequency, MFT=total mount frequency preceding for 30-min test, IFT=total intromission frequency for 30-min test, TCE=total copulatory efficiency for 30-min test.

[a] $P < 0.05$ significantly different than vehicle (Student' t test)

between the groups in the first test session while EL1 was significantly decreased in the rats administered with agomelatine in the second test session ($P < 0.05$, Table 1). Mount and intromission latencies were not significantly different from each other between
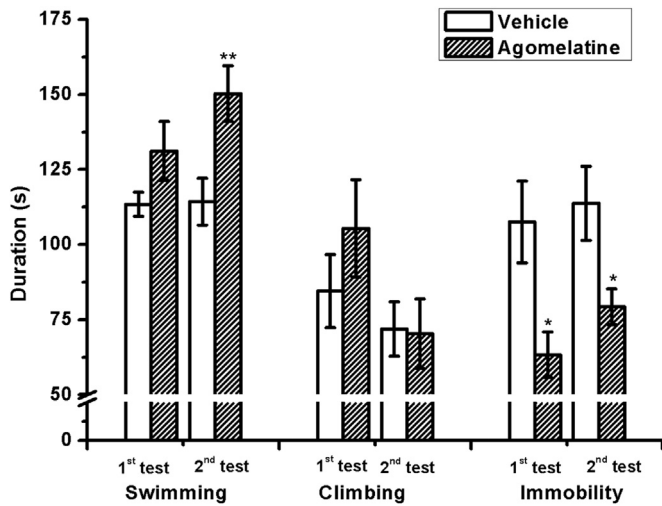
Fig. 1. Effects of chronic agomelatine treatment (14 weeks, 10 mg/kg.d) on antidepressant-like activity in the rat forced swim test (FST). Values plotted are mean ± S.E.M. *$P < 0.05$, vs. control group (Student's t test). $n = 10$ for each group.

the agomelatine-treated and control groups. Agomelatine treatment did not affect postejaculatory intervals. Although copulatory efficiency was significantly decreased ($P < 0.05$, Table 1) by agomelatine treatment in the first test session, there was no significant change in the second test session. Total copulatory efficiencies for 30-min test did not also show significant changes between the groups in the first and second test sessions.

3.2. Effects of agomelatine on the forced swimming test

The values regarding effects of agomelatine on the FST are seen in Fig. 1. Chronic administration of agomelatine induced a statistically significant decreases in duration of immobility in both of the FST tests ($P < 0.05$). The rats treated with agomelatine spent more time in swimming than the controls in the second FST test ($P < 0.01$). Swimming time was also higher in the agomelatine-treated rats but did not reach to a statistically significant value in the first FST test. Climbing time was statistically unchanged by agomelatine administration although it showed a tendency to be increased by agomelatine compared to vehicle-treated animals in the first FST.
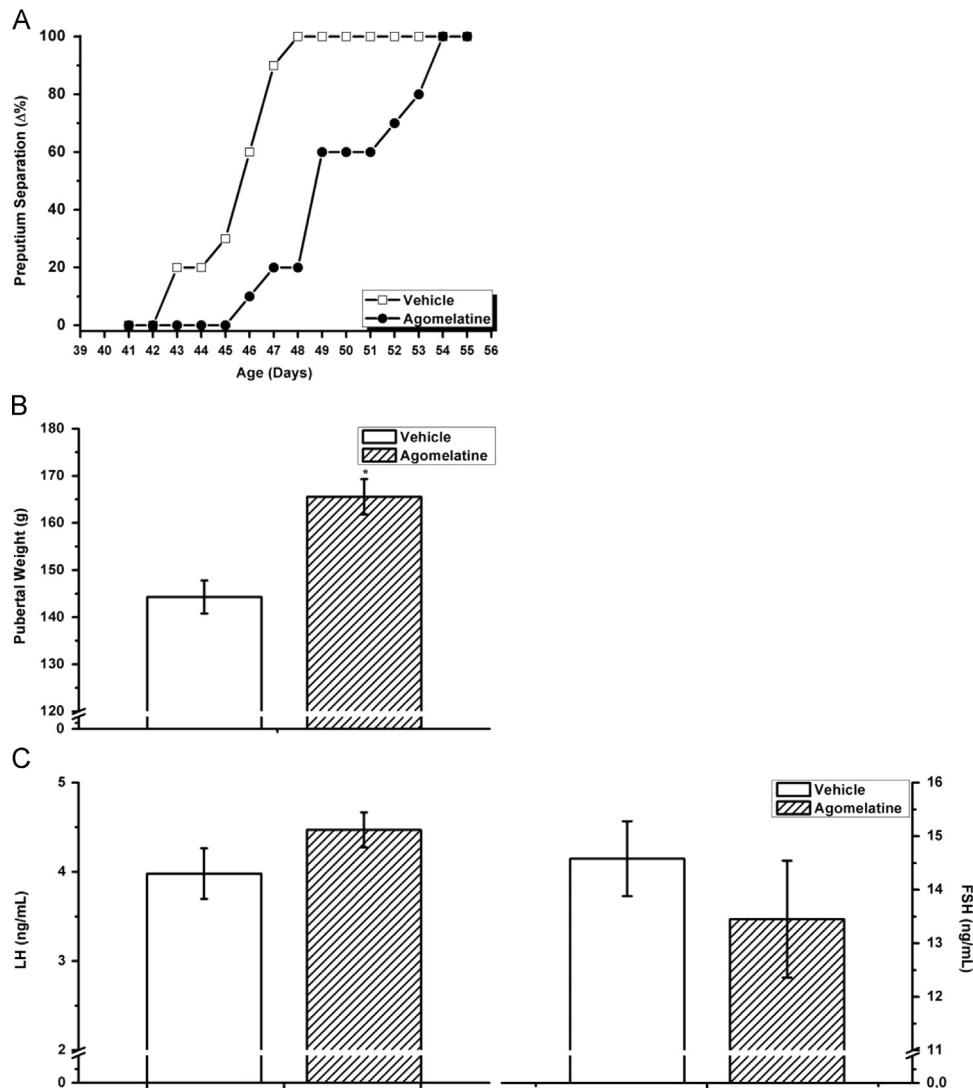

Fig. 2. Effects of chronic agomelatine treatment (14 weeks, 10 mg/kg.d) on different indices of puberty onset are documented in the male rats (A) preputial separation (B) pubertal weights (c) serum LH and FSH levels are presented for animals treated with vehicle and agomelatine. *$P < 0.05$; vs. control (vehicle) group (Student's t test). $n = 10$ for each group.

Table 3
Effects of chronic administration of agomelatine on reproductive organs in Spra-gue-Dawley rats – pnd 90 in the female rats and pnd 120 in the male rats.

	mg/100 g body weight	Control	Agomelatine	P-value
Male				
	Testicle	932.3 ± 61.46	818.7 ± 46.08	0.157
	Epididymis	451.6 ± 34.01	373.2 ± 24.61	0.078
	Seminal vesicle	183.2 ± 7.68	181.8 ± 16.90	0.942
	Prostate gland	147.1 ± 9.17	136.9 ± 12.72	0.521
Female				
	Ovary	55.9 ± 1.2	70.7 ± 4.2[a]	0.003
	Uterus	139.4 ± 7.1	176.6 ± 6.7[a]	0.001

Data is represented as mean ± S.E.M.

[a] $P < 0.005$ significantly different than vehicle (Student's t test)

3.3. Effects of agomelatine on pubertal maturation and reproductive organs

In the male rats, puberty onset was significantly delayed by agomelatine treatment compared with the control rats ($P < 0.001$, Fig. 2A). Pubertal weight was significantly higher in the agome-latine-treated group compared with control rats ($P < 0.001$, Fig. 2B). The reproductive organ weights were not altered by agomelatine treatment. The weights of testicle, epididymis, seminal vesicle and prostate gland were not significantly affected by agomelatine treatment (Table 3). The LH (4.47 ± 0.19 vs. 3.98 ± 0.28 for control group) and FSH (13.45 ± 1.09 vs. $14.5.19 \pm 0.69$ for control group) levels did not show any sig-nificant changes between the groups (Fig. 2C). Cauda epididymal sperm count was significantly lower in the agomelatine-treated rats compared with control rats (55.00 ± 4.7 and $73.3 \pm 4.3 \times 10^6$/organ, respectively, $P < 0.05$). Sperm motility was significantly lower in the agomelatine-treated animals than that in the control rats (78.3 ± 1.1 and $84 \pm 05\%$ respectively, $P < 0.001$).

Unlike in the male rats, chronic oral administration of agome-latine to the female rats advanced VO compared with control rats (43.1 ± 0.71 days vs. 48.9 ± 1.13 days for he control group, re-spectively, $P < 0.001$, Fig. 3A). Pubertal weight was found to be lower ($P < 0.05$) in the agomelatine-treated female rats compared to control rats (Fig. 3B). Uterine weight was significantly increased by agomelatine treatment compared with the control group
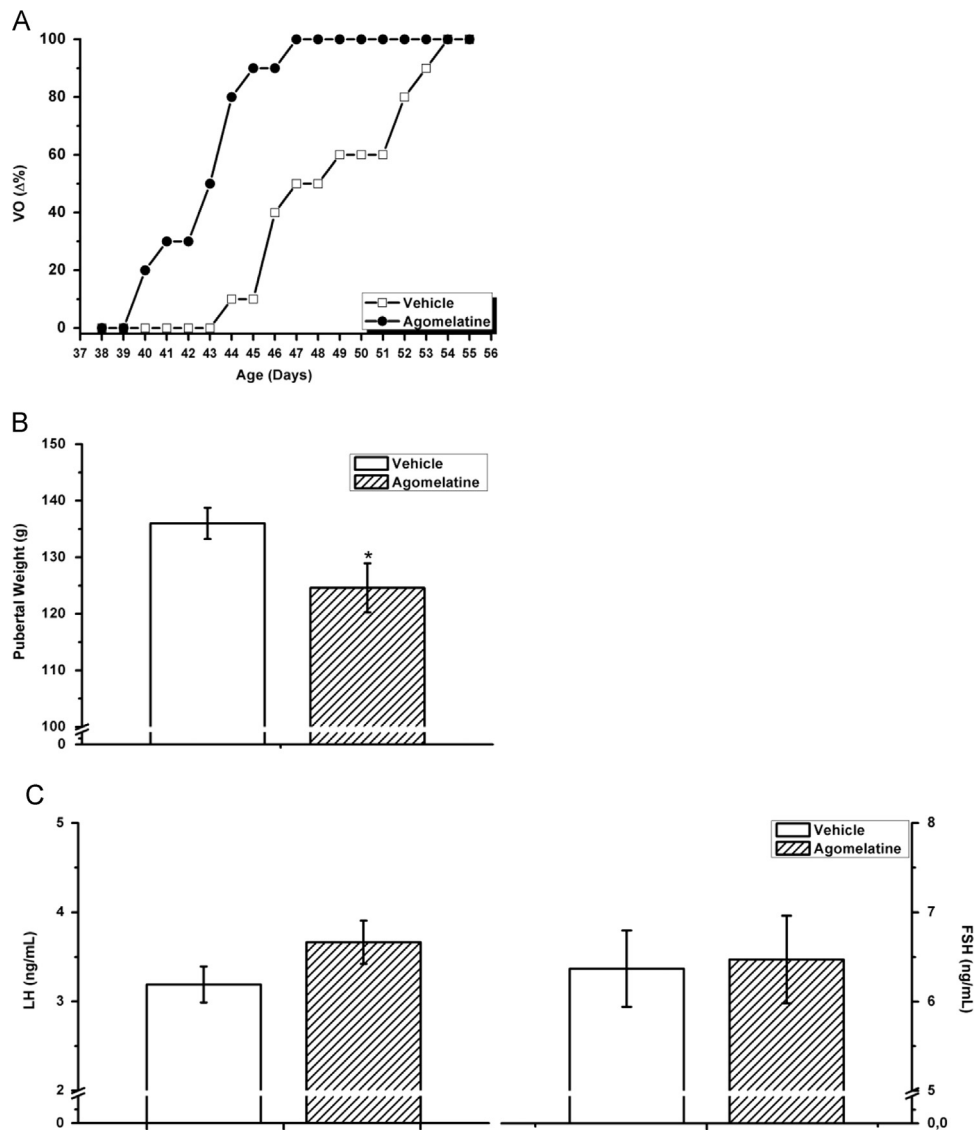

Fig. 3. Effects of chronic agomelatine treatment (14 weeks, 10 mg/kg.d) on different indices of puberty onset are documented in the female rats (A) vaginal opening (B) pubertal weight (c) serum LH and FSH levels are presented for animals treated with vehicle and agomelatine. *$P < 0.05$, vs. control (vehicle) group, (Student's t test). VO; vaginal opening. $n = 10$ for each group.

($P < 0.001$,Table 3). Ovarian weight was also significantly increased by agomelatine ($P < 0.01$,Table 3). Serum LH (3.66 ± 0.24 vs. 3.19 ± 0.2 ng/ml for the control group) and FSH (6.47 ± 0.49 vs. 6.36 ± 0.42 ng/ml for the control group) levels were not significantly affected by agomelatine treatment (Fig. 3C).

4. Discussion

4.1. Agomelatine and sexual behavior

Although there are at least a few studies on the effects of agomelatine on sexual function in humans, there is no study regarding the effects of agomelatine on sexual behavior in animal models to date. Therefore, this is the first study to investigate whether agomelatine has sexual side effects or not in rats. The present results shows that agomelatine maintained the stable character of the sexual behavior as indicated by the unchanged number of ejaculations. This finding is consistent with the concept that agomelatine does not cause marked sexual dysfunction in humans (Montejo et al., 2010). Moreover, intromission frequency was decreased in the agomelatine-treated rats, which suggests a facilitation of male sexual behavior induced by agomelatine treatment. So, the present results suggest that agomelatine has pro-sexual activity in the male rats. Since agomelatine is an agonist and antagonist for melatonergic receptors (MT_1 and MT_2) and $5\text{-}HT_{2C}$ receptors, respectively, the effects of agomelatine on sexual behavior may result from these different receptor properties. The fact that melatonin was previously shown to stimulate male sexual behavior in rats (Drago et al., 1999; Drago and Busa', 2000) indicates a possible role of melatonergic receptors in the pro-sexual activity of agomelatine. The male rats treated with melatonin were reported to have increased ejaculation frequency, decreased mount frequency and unchanged intromission frequency, which also suggests a facilitatory role of melatonin in sexual behavior like agomelatine (Brotto and Gorzalka, 2000). In that study, it is suggested that $5\text{-}HT_{2A}$ receptor may be involved in the action mechanism of melatonin. Thus, serotonergic receptors to which agomelatine and melatonin show antagonism seem to be different, $5\text{-}HT_{2C}$ and $5\text{-}HT_{2A}$ for agomelatine and melatonin, respectively. Different kinds of sexual responses to agomelatine and melatonin as indicated by ejaculation frequency may result from involvement of different types of serotonergic receptors. Since $5\text{-}HT_{2C}$ is suggested to elevate ejaculatory threshold (de Jong et al., 2006), agomelatine-dependent antagonism to $5\text{-}HT_{2C}$ receptor may lead to a decreased ejaculatory threshold without affecting the number of ejaculations. Agomelatine (14 days) has been reported to induce an an exitatory effect on dopamine neurons in the ventral tegmental area by blocking $5\text{-}HT_{2C}$ receptors (Chenu et al., 2013). Since dopamine is suggested to be facilitative to copulation (Hull et al., 1999), agomelatine may exerts its facilitator role in sexual behavior by modulating dopaminergic system. Agomelatine was previously reported to have an higher affinity for $5\text{-}HT_{2B}$, which was initially accepted not to be present in the brain, than $5\text{-}HT_{2C}$ receptors (Gobert et al., 2000). $5\text{-}HT_{2B}$ receptors have been now found to be present in the brain (Diaz et al., 2012). It has been suggested that the effect of agomelatine on the dopaminergic activity of ventral tegmental area (VTA) is enhanced when $5\text{-}HT_{2B}$ receptors, which may also produce an inhibitory effect on ejaculation (Yonezawa et al., 2008), are blocked by agomelatine (Chenu et al., 2014). Sleep loss has been suggested to promote marked changes in the male reproductive system of rats (Alvarenga et al., 2015). Therefore, agomelatine is likely to find many applications in sleep loss-induced sexual disorders because of its chronobiotic effect.

4.2. Effects of agomelatine on the forced swimming test

Despite the fact that chronic administration for the treatment of depression is needed to produce clinical effects, there is no study comparing the effects of chronic agomelatine treatment more than 4 weeks on behaviors in the FST in animal models. In the present experiment, agomelatine treatment continued from pnd 21 to pnd 120 about 14 weeks compared to a few weeks in the literature. Thus, this long term treatment has included both adolescence and adult periods. The present study indicates that chronic agomelatine treatment produces an anti-immobility activity in the FST in male rats. Like a wide range of antidepressant treatments (Cryan and Mombereau, 2004), the agomelatine-treated rats decreased significantly duration of immobility compared to the vehicle-treated rats in the test. Our findings are consistent with the previous studies showing decreased immobility following 22 day agomelatine treatment with a dose of 40 mg/kg i.p. (Ladurelle et al., 2012) and 13 day agomelatine treatment with the doses of 2, 10, 50 mg/kg p.o. (Bourin et al., 2004). In addition to immobility time in the FST in rats, there is only one agomelatine study (Ladurelle et al., 2012) looking at swimming and climbing behavior, which do not show statistically significant differences in the agomelatine-treated rats and control animals. In the current experiment, swimming time was significantly increased by chronic agomelatine treatment but climbing time remained unchanged despite showing a tendency to increase. Swimming behavior and climbing behavior are reported to be increased by serotonergic and noradrenergic antidepressants in rats, respectively (Cryan et al., 2005). Thus, the present findings suggest that agomelatine exerts an anti-immobility effect similar to that of SSRI in the FST. It is also concluded that agomelatine shows a stronger anti-immobility activity in the FST when chronically performed. Although, the previous studies suggest that the efficacy of agomelatine in the FST involves a combination of both its melatonin agonist and $5\text{-}HT_{2C}$ receptor antagonist properties (Bourin et al., 2004), their contribution ratios to antidepressant effect remain obscure. The antidepressant-like effects of agonist activity of agomelatine on melatonergic receptors are consistent with data reporting that melatonin reduced immobility times (Shaji and Kulkarni, 1998) but conflict with data showing that the rats treated with melatonin spent less time swimming and more time climbing (struggling) without no alteration in immobility behavior (Brotto et al., 2000).

4.3. Effects of agomelatine on reproductive functions

It has been demonstrated that melatonin treatment in children can be sustained over a long period of time without substantial deviation of the development of children with respect to puberty development (van Geijlswijk et al., 2011). Interestingly, agomelatine treatment from weaning had different effects on puberty onset in the female and male rats in our study. Agomelatine administration advanced puberty in the female rats while causing a delay in the male rats. Melatonin is generally accepted to have a puberty-delaying effect as the melatonin levels decrease considerably near puberty (de Holanda et al., 2011). Our previous study indicated that melatonin had a direct effect on immortalized GnRH GT1-7 cells (Kelestimur et al., 2012). The present finding that agomelatine advanced puberty onset in the female rats is not consistent with the results of the studies suggesting a puberty-delaying effect of melatonin. Therefore, there must be a mechanism by which agomelatine exerts its effect rather than centrally. Agomelatine treatment caused a reverse effect on puberty onset in the male rats compared to the female rats. Puberty onset was significantly delayed in the agomelatine-treated male rats. Melatonin is suggested to have an inhibitory role in Leydig cell steroidogenesis (Frungieri et al., 2005). Therefore, local effects of melatonin on testes may be responsible for its puberty-delaying effect.

One of the most interesting finding in the current experiment was that long-term administration of agomelatine caused

significant decreases in sperm count and motility. Since agomelatine has been reported to increase serum prolactin level (Tan, 2013), the decrease in spermatogenesis may result from the changes in the prolactin secretion. The changes in sperm quality resulting from chronic agomelatine treatment do not seem to impair fertilization process (Cooper et al., 2010). Further studies looking at the effects of agomelatine on human sperm quality should be carried out as agomelatine has been suggested not to have any effect on semen quality (Elnazer and Baldwin, 2014), which is a case report including only one patient.

In conclusion, chronic treatment with agomelatine does not lead to adverse effects on sexual behavior and even exerts some putative pro-sexual effects in adult male rats. To our knowledge, this study provides the first evidence of lack of sexual dysfunction by chronic agomelatine treatment in rats. Our work also shows that agomelatine induces an antidepressant-like effect by means of enhancement of serotonergic and possible dopaminergic activity. Further studies are needed to explore whether agomelatine shows an antidepressant-like effect without sexual dysfunction in female rats. Finally, as our data suggest that the effects of chronic agomelatine administration on reproductive seem to be sex-dependent, clinical use of agomelatine may lead to different side-effects in male and female. The understanding of the effects of agomelatine will not only contribute to the delination of the combination of melatonergic and 5-HT$_{2C}$ receptor activation in the treatment of depression, but may also provide new therapeutic approaches to treat some reproductive disorders.

Acknowledgments

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