Research report

Anxiolytic-like actions of reboxetine, venlafaxine and endurance swimming in stressed male rats

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\textbf{A B S T R A C T}

Despite being potent anxiolytic agents, benzodiazepines (BDZ) sometimes show reduced therapeutic efficacy in stressed rodents. However, the effectiveness of norepinephrine reuptake inhibitors (NRI) and serotonin–norepinephrine reuptake inhibitors (SNRI) or other anxiolytic interventions, e.g., exercise, remained elusive. Here, we demonstrated that male rats subjected to restraint stress for 4 weeks showed decreases in percent open arm time and open arm entry, as determined by elevated plus-maze test (EPM). Increases in inhibitory avoidance trial 2 and outer zone time were also observed in elevated T-maze (ETM) and open field test (OFT), respectively. To evaluate the anxiolytic-like actions of exercise and anxiolytic drugs, stressed rats were subjected for 4 weeks to swimming or daily gavage with 2 mg/kg diazepam (BDZ), or 10 mg/kg fluoxetine (selective serotonin reuptake inhibitor), reboxetine (NRI), or venlafaxine (SNRI). In EPM, the open arm activity was higher in the swimming, reboxetine-treated and venlafaxine-treated groups as compared to age-matched controls, while diazepam and fluoxetine were without effect. In ETM, a reduction in avoidance latency was observed only in swimming and venlafaxine-treated groups. However, the combined swimming and pharmacological treatment showed no additive anxiolytic-like effect. It could be concluded that restraint stress induced anxiety-like behaviors, which were not responsive to diazepam or fluoxetine, whereas reboxetine and venlafaxine and swimming showed anxiolytic-like actions in stressed rats.

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1. Introduction

Anxiety disorders, which are usually aggravated by stress, are common psychiatric problems with an estimated lifetime prevalence of ~17% [1]. In rodents, chronic stress often contributes to the progress of anxiety and depression, leading to excessive physiological and/or behavioral responses to fear [2]. At the cellular level, stress induces neuronal atrophy and cell loss in several brain regions related to mood disorders, e.g., hippocampus, amygdala and prefrontal cortex [3]. Although pharmacological treatments by benzodiazepines (BDZ) that target brain \(\gamma\)-aminobutyric acid (GABA)-ergic system have been proven effective in anxiety disorders [4], BDZ sometimes showed decreased effectiveness in stressed rodents with increased anxiety [5]. Whether non-BDZ anxiolytic drugs could alleviate anxiety-like behaviors in stressed male rats remained elusive.

A number of neurochemical modulators, especially norepinephrine (NE) and serotonin (5-hydroxytryptamine; 5-HT), are involved in anxiety disorders [6]. For example, abnormal serotonergic functions caused by dysregulation of 5-HT release and reuptake or decreased responsiveness to serotonergic signal lead to anxiety disorders [7]. Thus, selective serotonin reuptake inhibitors (SSRI), such as fluoxetine, are potent drugs for anxiety disorders [8]. Furthermore, since NE as a sympathetic modulator has a prominent role in anxiety responses induced by chronic stress exposure [9], we postulated that norepinephrine reuptake inhibitors (NRI; reboxetine) and serotonin–norepinephrine reuptake inhibitors (SNRI; venlafaxine) could be used as the effective agents to treat anxiety in stressed individuals.

Besides pharmacological treatments, regular endurance exercise—aerobic exercise that is done at moderate intensity to increase stamina—has been reported to have anti-depressive and anti-anxiety effects in patients with depression and anxiety disorders [10–12]. Animal study also showed that impact exercises—aerobic exercises, such as treadmill and voluntary wheel running, that cause impact on bones and joints—exhibited anxiolytic-like effect on the rodent behaviors as evaluated by
various behavioral assessments. For example, Fulk et al. [13] demonstrated that moderate treadmill running decreased anxious behaviors of rats in elevated plus-maze test (EPM) and open field test (OFT). In addition, long-term wheel running reduced anxiety-like behaviors in mice with asthmatic stator, stress-induced hyperthermia, social interaction and light-enhanced startle test [15]. We, therefore, hypothesized that endurance exercise may help mitigate anxiety-like symptoms in stressed animals.

In the present study, we used endurance swimming as a model of exercise intervention. As a non-impact exercise, swimming has been a widely used intervention in overweighted and osteoporotic patients, or even in healthy individuals because, in contrast to impact exercise, it does not cause bone and joint injury. Although the anxiety-like effect of swimming in stressed animals remained elusive, a behavioral study in chronic stressed rats revealed positive effect of 4-week swimming on exploratory activity in OFT and 5-HT metabolism in the brain [16]. Furthermore, moderate-intensity swimming markedly improved the locomotion and hypothalamic-pituitary-adrenal (HPA) axis function in stressed rats [17]. The aforementioned findings thus suggested that swimming could help alleviate anxiety-like behaviors in stressed rats.

Therefore, the principal objectives of the present study were (i) to investigate the efficiency of each anxiolytic drug treatment (i.e., BDZ, SSRI, NRI and SNRI) and endurance swimming in the reduction of anxiety-like behaviors in stressed male rats by using elevated T-maze test (ETM), EPM and OFT; and (ii) to determine the anxiolytic-like actions of combined drug treatment and swimming in stressed male rats.

2. Materials and methods

2.1. Animals

Eight-week-old male Wistar rats (weighing 180–220 g) were obtained from the National Animal Centre of Thailand, Mahidol University, Salaya Campus, Thailand. Animals were housed in stainless-steel shoebox cages with wire covers (24 cm × 48 cm × 18 cm; 2 rats/cage) at 25 ± 2°C and 55 ± 5% humidity under 12:12-h light–dark cycle (light on from 06:00 h to 18:00 h; average illuminance 200±1 lx). All rats were fed standard chow (CP Company, Ltd., Thailand) and water ad libitum, and first acclimatized in the vivarium for at least 7 days before the start of the experiments. Ten rats (n = 10) were used in each experiment. All animals were cared for in accordance with the Guide for the Care and Use of Laboratory Animals, National Research Council (1985 edition). This study has been approved by the Animal Care and Use Committee of the Faculty of Medicine, Thammasat University, Thailand.

2.2. Experimental design

After 7-day acclimatization, a series of experiments (Fig. 1A) was performed to determine the anxiety-like behaviors in stressed male rats (restraint stress 1 h/day, 5 days/week for 4 weeks; performed between 08:00 and 09:00 h) as compared to their age-matched non-stressed controls. To investigate the anxiolytic-like actions of each anxiolytic drugs (Fig. 1B), stressed rats were once-daily administered through a 18-gauge gavage tube for 4 weeks (7 days/week) with 5 mL/kg vehicle (normal saline), 2 mg/kg diazepam (BDZ), 10 mg/kg fluoxetine hydrochloride (SSRI), 10 mg/kg reboxetine methanesulphonate (NRI), and 10 mg/kg venlafaxine hydrochloride (SNRI). Commercial tablets of diazepam (10 mg active drug/tablet; Government Pharmaceutical Organization, Bangkok, Thailand), fluoxetine (20 mg active drug/tablet; Eli Lilly, Indianapolis, IN, USA), reboxetine (4 mg active drug/tablet; Pharmacia & Upjohn, Milan, Italy), and unpacked venlafaxine particles (75 mg active drug/capsule; Wyeth-Whitehall, Ireland) were ground to obtain fine powder. All drugs were prepared fresh daily by being dissolved in normal saline solution to obtain a final gavage volume of 5 mL/kg. Drugs were administered at 16:00 h after restraint stress session. The present dosage and treatment protocols were based on the optimal doses reported previously [18–21]. Since all rats were subjected to gavage procedure, the data could be interpreted without a bias from gavage-induced stress.

At the end of the anxiolytic-like effect of endurance swimming (Fig. 1B), stressed rats were divided into 2 groups, i.e., swimming and age-matched sedentary control groups. The restraint procedure was performed in a quiet room between 08:00 and 09:00 h before exercise session (5 days/week for 4 weeks, 1 h/day; 15:00–16:00 h on Monday to Friday). In the swimming group, the 7-day initial swim training was required to familiarize rats with water. On the other hand, the sedentary rats were placed in shallow water for the same duration as the initial swim training and endurance swimming periods. Moreover, to minimize variation due to the age of rats, all experiments commenced (Experimental day 0) when the rats were 10 weeks old as depicted in Fig. 1B.

For the combined drug/exercise treatments (Fig. 1C), rats were subjected to stress induction during 08:00–09:00 h (5 days/week for 4 weeks), followed by endurance swimming at 15:00–16:00 h. The stressed swimming rats were administered orally with various anxiolytic drugs (at 16:00 h, 7 days/week for 4 weeks), as described in the preceding experiment.

As depicted in Fig. 1, on day 28 of the experiments, all rats were subjected to three anxiety-related behavioral tests (i.e., EPM, ETM and OFT) between 6:00 and 12:00 h (i.e., 24 h after the last stress exposure or 16 h after the last pharmacological treatment). Each rat was brought to the test room and left undisturbed in a quite environment for at least 5 min. The room was kept dim with average illuminance of 20 lx. EPM was first performed, followed by ETM and OFT on the same day (5-min interval between tests). Rat behaviors were recorded in dim light by an infrared video camera (model HDR-XR200E; Sony, Tokyo, Japan) suspended above the testing apparatus. The apparatus was thoroughly cleaned after each test with wet towel and 70% ethanol to eliminate odorants and residues (e.g., feces and urine) that could affect behavioral responses. Rats were always handled by the same researcher to minimize handling stress throughout the 7-day acclimatization and 4-week experimental periods. Each rat experienced each behavioral test only once prior trials to avoid learning which could render behavioral data inaccurate. During the test, each rat was subjected to the test one at a time with no others in the same room.

At the end of swimming experiments (day 28), the heart was removed from the euthanized rat, washed with ice-cold normal saline solution and blotted dry with filter paper. Wet weight of the heart was then recorded. Thereafter, the heart was dried in an incubator at 80 °C for 3 days to obtain the dry heart weight. Both wet and dry heart weights were used to indicate the effectiveness of endurance swimming [22].

2.3. Stress induction

The present stress induction protocol was modified from the method of Ely et al. [23], and is one of the widely used stress-inducing paradigms involving immobilization of the animals. In brief, each rat was individually restrained by being placed in a tightly fitted plastic cylinder (24 cm long, 6 cm inner diameter with one closed end) for 1 h/day, 5 days/week for 4 weeks. Therefore, stressed rats could not move freely. There was a 1-cm aperture at the closed end for breathing.

2.4. Endurance swimming

The present swim training is a model for non-impact endurance exercise with moderate intensity, which successfully induces cardiac hypertrophy and enhances citrate synthase activity in the skeletal muscle without accumulation of lactate in the plasma [22]. Briefly, the rats in swimming groups were assigned to perform endurance swimming for 4 weeks, while the age-matched control rats remained sedentary in the swimming chamber filled with shallow water (30–32 °C tap water, 5 cm deep; all four paws reached the bottom of swimming chamber). The swimming chamber was made of transparent Plexiglas cylinder with a dimension of 80-cm high and 45-cm inner diameter, filled with tap water 45-cm deep. The water temperature was strictly maintained at 30–32 °C and none of animals experienced hypothermia, which could be determined with additional water temperature sensor. Swimming rats were initially trained for 7 days (i.e., initial training period; starting at 10-min duration on experimental day 7 with 10 min/day being added on each day: Fig. 1) until they were capable of swimming nonstop 1 h/day. During the endurance swimming period (experimental days 0–28), swimming frequency was 5 days/week (1 h/day; 15:00–16:00 h on Monday to Friday).

2.5. Elevated plus-maze test

The maze was made of wood painted black and elevated 50 cm above the ground. The apparatus consisted of two open arms (50 cm × 10 cm) aligned perpendicularly to two closed arms (50 cm × 10 cm × 40 cm). The open arms had a 1-cm high Plexiglas rim to prevent fall. Each rat was gently placed onto the central square of the apparatus, facing open between open arm and closed arm. The animal was allowed to explore the apparatus for 5 min, while its behavior was recorded by an infrared video camera. Behavioral responses determined in this study were the time spent in open and closed arms, number of entries into open and closed arms, rearing, and grooming. Arm entry was counted when all four paws entered the arm. Increased time spent in open arm, number of entries into open arms and/or rearing were indices of anxiety, whereas the numbers of total and closed arm entries indicated general locomotor activity [24,25].

2.6. Elevated T-maze test

The black wooden T-maze consisted of three arms with equal dimension (50 cm × 10 cm). The closed arm was enclosed by walls (40 cm high), situated perpendicularly to two opposed open arms. These three arms were connected by the
Fig. 1. Timelines show the present experimental design, i.e., (A) the anxiety-like behaviors in stressed rats, (B) anxiolytic-like effects of pharmacological treatment or exercise intervention in stressed rats, and (C) anxiolytic-like effects of combined drug treatment and exercise intervention in stressed rats. Eight-week-old rats were first acclimatized for 7 days in the vivarium. In the anxiolytic drug-treated, vehicle-treated or stress-only groups without exercise, rats were housed in their cages for additional 7 days in order to start experiments at the same age as the exercise groups. In the experimental groups with swimming, the 7-day initial swim training was performed after acclimatization to familiarize swimming rats with water and to ensure that they could swim nonstop for 1 h/day. Sedentary rats that were used as controls in the swimming experiments were placed in the swimming chamber containing 5-cm water (during both initial swim training and endurance swimming periods). Induction of restraint stress and drug administration commenced on experimental day 0. Finally, all rats were subjected to three behavioral tests. For more details, please see Section 2.

central square (10 cm × 10 cm). The maze was also elevated 50 cm above the ground. To prevent fall, the open arms were guarded by 1-cm high Plexiglas rims. This behavioral test was composed of three inhibitory avoidance trials (i.e., baseline, avoidance 1 and avoidance 2) and one-way escape trial held at 30 s intervals. In the inhibitory avoidance trials, each rat was placed at the distal end of the enclosed arm, facing the central square of the maze. The baseline duration (in seconds) was determined as the time taken to withdraw from this arm with all four paws. Thereafter, the procedure was repeated for two additional avoidance trials (avoidance 1 and 2). After the avoidance trials, an escape trial was performed by placing rat at the distal end of the right open arm, facing the central square of the maze. The escape latency was
the duration (in seconds) taken to exit this arm and enter the closed arm with four paws [26]. In general, the inhibitory avoidance represents conditioned fear, while one-way escape from the open arm represents unconditioned fear [27].

2.7. Open field test

The open field apparatus was made of a black wooden box (76 cm long × 57 cm wide × 35 cm high) with a 48-square grid floor (6 × 8 squares; 9.5 cm per side). The arena was divided into two zones, i.e., the inner and outer zones (24 peripheral squares each). Each rat was gently placed in one of the four-corner squares of the apparatus for 5 min. The locomotor performance and behavioral responses were recorded by an infrared video camera. The obtained behavioral parameters were the number of lines crossed in the first 30 s, total number of lines crossed, and time spent in the inner and outer zones of the arena [26]. Line crossing was counted when all four paws of rats had crossed the line marked on the floor. The lines crossed during the first 30 s of the test indicated an exploratory behavior for the novelty. The total number of lines crossed was a determinant of the overall locomotor activity [28]. An increase in time spent in the outer zone (or thigmotaxis) and/or a decrease in time spent in the inner zone was indicative of anxiety-like behavior in rodents [29].

2.8. Statistical analyses

The results are expressed as means ± SE. Comparisons between the two data sets were performed by unpaired Student’s t-test, whereas multiple comparisons were performed by one-way analysis of variance (ANOVA) with Newman–Keuls’ post-test. The t-values, F-values and degree of freedom (df) values were also reported in the figures. The level of significance was P < 0.05. All statistical tests were performed by GraphPad Prism 5.0 (GraphPad Software Inc., San Diego, CA, USA).

3. Results

3.1. Restraint stress induced anxiety-like behaviors in male rats

As determined by EPM, stressed male rats showed lower percent open arm time and percent open arm entry than the control rats, whereas other parameters, i.e., closed arm entry, total entry, and numbers of rearing and grooming remained unchanged (Fig. 2A). ETM further revealed that stressed rats exhibited higher avoidance 2 latency as compared to the non-stressed control group (Fig. 2B). However, the 4-week restraint stress did not alter the baseline, avoidance 1 and one-way escape latencies (Fig. 2B). Moreover, stressed rats also spent more time in the outer zone and less time in the inner zone of the open field arena when compared to the non-stressed control rats (Fig. 2C). Restraint stress did not change the numbers of the 1st 30 s lines crossed (Fig. 2C) and total lines crossed (Fig. 2C), which were indicators of exploratory behavior and locomotor activity, respectively. The results suggested that the 4-week restraint stress induced anxiety-like behaviors in male rats, especially the learned fear as indicated by a marked increase in avoidance 2 latency.

3.2. Reboxetine, venlafaxine and endurance swimming showed anxiolytic-like action in stressed male rats

As compared to the corresponding age-matched sedentary rats (n = 10), the vehicle-treated stressed rats (n = 10) subjected to the 4-week endurance swimming showed increases in wet heart weight [1.08 ± 0.03 vs. 1.16 ± 0.04 g; t(18) = 1.816; P = 0.043], body weight-normalized wet heart weight [2.87 ± 0.09 vs. 3.11 ± 0.10 × 10⁻³; t(18) = 1.885; P = 0.038], dry heart weight [0.28 ± 0.01 vs. 0.31 ± 0.01 g; t(18) = 2.332; P = 0.016], and body weight-normalized dry heart weight [0.74 ± 0.03 vs. 0.82 ± 0.02 × 10⁻³; t(18) = 2.446; P = 0.013]. This exercise-induced cardiac hypertrophy was similarly observed in the anxiolytic drug-treated stressed swimming rats (data not shown).

In stressed male rats subjected to EPM (Fig. 3), reboxetine and venlafaxine treatment led to higher percent open arm time when compared to vehicle treatment. However, only venlafaxine significantly increased number of open arm entries (F₂₄,₄₅ = 4.217; P = 0.006; n = 10 per group), percent open arm entry (Fig. 3B) and number of rearing (Fig. 3D), without changes in the closed arm entry (Fig. 3C), total arm entry, or number of grooming (data not shown). No behavioral parameter from EPM was changed by diazepam and fluoxetine (Fig. 3). Interestingly, stressed rats subjected to endurance swimming for 4 weeks showed higher number of open arm entries [2.40 ± 0.45 vs. 4.70 ± 1.04 times; t(18) = 2.022; P = 0.029; n = 10 per group], and percent open arm entry (Fig. 3B) than the age-matched sedentary rats.

In EPM, only venlafaxine treatment resulted in lower baseline, avoidance 1 and 2 latencies as compared to the vehicle treatment, whereas stressed swimming rats showed lower avoidance 1 and 2 latencies than the age-matched sedentary rats (Fig. 4). Neither anxiolytic drugs nor endurance swimming altered one-way escape latency in the EPM (Fig. 4D) as well as outer and inner zone times, 1st 30 s lines crossed, or total lines crossed in the OFT (Supplementary Figure S1). These results indicated that the two pharmacological treatments (reboxetine and venlafaxine) and exercise intervention (endurance swimming) did have anxiolytic-like actions in stressed male rats.

Supplementary material related to this article found, in the online version, at doi:10.1016/j.bbr.2012.02.037.

3.3. Combined drug/swimming had no synergistic anxiolytic-like effect on stressed rats

In the last series of experiments, anxiety-like behaviors were determined in stressed rats subjected to a combined anxiolytic drug treatment and swimming. As shown in Supplementary Figures S2–S4, no drug regimen altered anxiety-related parameters in stressed swimming rats when compared to the vehicle treatment, suggesting that the anxiolytic drugs and endurance swimming had no synergistic anxiolytic-like effect under the present restraint stress condition.

Supplementary material related to this article found, in the online version, at doi:10.1016/j.bbr.2012.02.037.

4. Discussion

The present 4-week restraint stress was found to induce anxiety-like behaviors in male rats, particularly learned fear as clearly indicated by an increase in avoidance 2 latency in the EPM. Although some commonly used anxiolytic drugs, such as diazepam and fluoxetine, could not alleviate the anxiety-like behaviors in these animals, our investigation found that reboxetine and venlafaxine were effective. Besides the pharmacological treatment, endurance swimming as an exercise intervention also had an anxiolytic-like action in these stressed rats.

Like other mild- to moderate stressors, restraint stress is known to activate HPA axis and adrenal corticosteroid secretion [30]. Prolonged corticosteroid release during stress eventually results in neuronal malfunction and anxiety [9], consistent with the present behavioral study in male rats that the 4-week restraint stress induced anxiety-like behaviors. Although not measured in this study, circulating adrenocorticotropic hormone (ACTH) and corticosterone levels were probably increased since the anxiety-like behavior in rats was associated with the plasma levels of ACTH and corticosterone [31].

In EPM, the stressed rats exhibited a decrease in open arm activity (Fig. 2A). They also manifested conditioned (learned) fear, which resembled generalized anxiety disorder in humans [27], as indicated by prolonged avoidance latency in EPM (Fig. 2B). Wood et al. [32] demonstrated that chronic immobilization stress affected emotional and learned aspects of fear task in the EPM, as evinced by a longer time spent in inhibitory avoidance task, expressed aggression, and struggling and helplessness behaviors. In OFT, an
Fig. 2. Behavioral responses of male rats to the 4-week restraint stress as determined by (A) elevated plus-maze (EPM), (B) elevated T-maze test (ETM), and (C) open field test (OFT). *$P<0.05$, **$P<0.01$ compared to its respective control group. Numbers in parentheses represent the numbers of experimental animals.
increase in ambulation in the outer zone (Fig. 2C) generally indicates anxiety-related behavior in rodents [29,33]. Thus, the present findings that stressed rats spent more time in the outer zone without defects in general locomotor activity or exploratory behavior confirmed that these stressed rats did have anxiety.

Since exposure to environmental stressors often altered 5-HT, NE, and dopamine (DA) levels in the brain, which in turn led to dysregulation of stress circuits [34], anxiolytic drugs which increased serotonergic, noradrenergic and/or dopaminergic neurotransmission could help mitigate stress-induced anxiety-like behaviors. This was consistent with the effectiveness of the NRI and SNRI anxiolytic drugs, i.e., reboxetine and particularly venlafaxine, in our stressed rats, whereas diazepam, a potent BDZ anxiolytic drug, was not. Indeed, diazepam was previously found to be less effective for reducing anxiety-like behaviors in stressed male rats [5] and tree shrews (Tupaia belangeri) [35]. However, our findings that fluoxetine was also ineffective in reducing anxiety-like behaviors in stressed rats contradicted previous reports of an anxiolytic-like action of fluoxetine in stressed rats [19,36,37]. Since antidepressive and anxiolytic actions of fluoxetine were usually seen after 4–6 weeks of treatment, the present 4-week study might be too short to demonstrate its full effect.

On the other hand, the beneficial effects of reboxetine and venlafaxine in stressed rats with anxiety were seen as early as 4 weeks in EPM and ETM. Neither treatment altered the thigmotaxis in OFT, suggesting that reboxetine and venlafaxine might affect only some aspects of stress-induced anxiety (e.g., learned fear in venlafaxine-treated group). Although reboxetine has been widely investigated for its antidepressant action, and has been reported to increase active behaviors in the forced swim test [38], its anxiolytic-like effect remained elusive. Garner et al. [8] recently suggested that reboxetine might be used in acute treatment of panic disorder. Hence, we demonstrated experimentally, for the first time, that reboxetine did have an anxiolytic-like action without effect on locomotor activity in stressed rats, as determined by the EPM and OFT, respectively. Furthermore, venlafaxine, an inhibitor of 5-HT and NE reuptake with superior clinical efficacy over other anxiolytic/antidepressant drugs due to its fast onset of action, was also found to effectively alleviate anxiety-like behaviors in stressed rats as indicated by increases in open arm activity and number of rearing in the EPM, and impaired inhibitory avoidance in the ETM. Similarly, de Oliveira et al. [21] also showed that venlafaxine-treated rats with sleep deprivation-induced stress manifested increases in the time spent in the open arms, number
of entries, crossing lines, and rearing in the EPM and OFT. Moreover, venlafaxine reversed the anxiogenic effect of chronic stress in rats as indicated by increases in the time spent in light compartment and crosses between compartments in the two compartment exploratory test [39]. However, further investigation is required to determine the underlying cellular mechanism of venlafaxine in stressed animals.

Besides pharmacological treatments, exercise intervention (endurance swimming) could also alleviate the anxiety-like behaviors in stressed male rats. Aerobic exercise training produces anxiolytic-like effects that can protect against harmful consequences of stress [11]. Exercise also has antidepressant- and anxiolytic-like effects in healthy subjects and patients with anxiety disorders [12]. For example, regular aerobic exercise despite being less effective than typical anxiolytic drug clomipramine could reduce the symptoms of panic disorder [40]. In addition, 3–12 weeks of exercise was reported to reduce anxiety symptoms in chronic illness patients [41]. The present finding that swimming rats showed an increase in open arm activity in the EPM and a decrease in avoidance latency in the ETM suggested that swimming did have an anxiolytic-like action in these rats. Moreover, in rats subjected to stress-induced anxiety, moderate-intensity swimming reduced freezing time in the hole-board test, as well as the stress-induced oxidative injury in several organs, e.g., heart, liver, stomach, and brain [42].

Indeed, the exact mechanism of anxiolytic-like actions of exercise in both humans and rodents is not well understood. It was suggested that exercise partly modulated brain function and provided neuroprotection in several neuropsychiatric disorders through neurogenesis and neural plasticity [43]. The beneficial effects of swimming on the brain included memory improvement, reduction of reactive oxygen species production, and enhanced production of BDNF and NGF for neurogenesis [44,45]. Based on the fact that reduced heat shock protein (HSP)-70 synthesis and elevated circulating corticosteroids are the hallmark of stress-related diseases, swimming probably exerted its anxiolytic-like action by modulating HSP70 and glucocorticoid receptor expression in the hippocampus [46]. A recent study also showed that swimming improved the emotional state of stressed rats in the OFT, prevented stress-induced change in the HPA axis, and induced HSP70 upregulation and inducible nitric oxide synthase (iNOS) downregulation in the limbic region [17]. In addition, increases in brain monoamine production (e.g., 5-HT, NE, and GABA) and receptor responsiveness helped alleviate the anxiety-like symptoms during exercise.
[47,48]. For example, treadmill training in male rats increased NE levels in pons, medulla oblongata and spinal cord, and enhanced NE metabolism in the frontal cortex and hippocampus [49]. The exercise-induced endorphin secretion and increased blood perfusion to the brain may also contribute to its antidepressant effect [50]. Nevertheless, further experiments are required to demonstrate the precise molecular mechanism of anxiolytic-like action of swimming in stressed rats.

Although both swimming and some anxiolytic drugs could individually alleviate anxiety-like behaviors in stressed male rats, combined drug treatment and swimming intervention did not show additive or synergistic effect (Supplementary Figures S2–S4). In female mice, wheel-running exercise did not synergize with fluoxetine in the induction of BDNF and insulin-like growth factor (IGF) production [51]. It was possible that exercise and these anxiolytic drugs shared the same underlying mechanism of their anti-anxiety action. Specifically, exercise and drug treatment might similarly enhance hippocampal neurogenesis, or production of endogenous opioids and other modulating substances [43–45,50]. Thus, synergistic effect was not observed in a combined study.

In contrast to the aforementioned advantages, long-term forced swimming (up to 8 weeks), high-intensity impact exercise or overtraining are themselves stressors, which could induce stress response and anxiety-like behaviors [52]. Therefore, it is necessary to design appropriate exercise protocol with maximal benefit for stressed individuals with anxiety otherwise exercise intervention may unintentionally aggravate their anxiety symptoms.

In conclusion, the 4-week anxiolytic drug treatments, i.e., reboxetine and particularly venlafaxine, but not diazepam and fluoxetine, appeared effective in alleviating anxiety-like behaviors in stressed male rats as determined by EPM and/or ETM. Endurance swimming as an exercise intervention also helped reduce anxiety-like behaviors, but showed no additive effect with pharmacological treatments. The absence of anxiolytic-like action of venlafaxine and exercise in OFT suggested that they might be effective only for some particular aspects of anxiety, such as learned fear, which could be revealed by ETM, but not OFT. However, further experiments are required to demonstrate the underlying cellular mechanisms by which reboxetine, venlafaxine and endurance swimming alleviate the stress-induced anxiety, and whether they are effective in stressed patients.

Conflict of interest

None declared.

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