The Antidepressant-like Action of Human and Caprine Amniotic Fluid in Rats: Effect of Gender

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Abstract Depression is a common worldwide burdening mental disease. The amniotic fluid (AF) in both humans and animals has been reported to be associated with reduction of anxiety, pain and with helping the newborns to recognize their mothers. As far as we know, no published work on its possible antidepressant action has been reported before. Therefore, we tested here if AF has an anti-depressant-like activity, and investigated if there is a gender difference in this action. Also, we wanted to find out if this possible action can be modified by a known antidepressant drug. Sixty male and female Wister rats were divided randomly into five groups in each gender, and treated with the following: water (control), human AF, goat AF, venlafaxine and venlafaxine+ human AF. AF and venlafaxine were given orally by gavage at a dose of 0.2ml/ Kg once a day for five days. Twenty four hours later, locomotor activity test, and the forced swimming test (FST) were measured. Motor activity and immobility time in the FST were both significantly reduced in male and female rats treated with human and caprine AF. Rats treated with human AF showed the least activity. The latter effects were not significantly affected by concomitant treatment with venlafaxine and human AF. The biochemical mechanisms of antidepressant like effect of AF warrant further study.

Keywords: depression, forced swimming test, amniotic fluid, motor activity, rats


1. Introduction

Depression is an affective disorder that has become a common health problem worldwide [12]. It was found to be common in adults (between 20-40 years), and also more common in women than men, with a double prevalence [6]. Depression has become the 4th main cause of Disability Adjusted Life Year (DALYS) which is used to measure the suffering of a population based on time lived with a disability. It was found that, about 5-10% of the population in the world suffer from a depressions symptom such as loss of hope and inadequacy, loss of interest or pleasure, sleep disturbance, poor concentration and lethargy there symptoms associated with depression require mental or psychiatric intervention [19].

The prevalence of depression in our region of the world (exemplified by Oman) is equally distributed between the two genders and that 17% of the sample studied had symptoms of depression which suggests that depression is a public health problem in this country [3], and that it is still not widely diagnosed or treated because of the masking effect of drug addiction, alcoholism or physical complaints [6].

It has been reported that there is definite effect when human newborns are exposed to AF odors, causing a calming action, and resulting in a reduction of pain, and also helping the child to recognize their mothers, and this explains why children cry when their mothers are absent [17]. This effect has also been observed with other mammals [9]. It is known that certain lipids (and other constituents) in plasma can be used as bio- markers for anxiety, depression, and fatigue [11].

Certain fatty acids in human amniotic fluid have recently been shown to possess anxiolytic – like action in animal models [16]. However, and as far as we are concerned, nothing has been published on the possible action of AF on depression in humans or animals. Therefore, the aim in the current work was to test the effect of orally administered AF (from healthy humans or goats) on the forced swimming test (FST), an established model of depression, and their possible interaction with a clinically useful antidepressant drug, venlafaxine.

2. Methods

2.1. Animals

The experimental groups consisted of 30 male and 30 female two months-old Wister rats, each weighing about 200g. They were individually housed six in a cage, at a
temperature of 22 ± 2 °C, relative humidity of 50–60% and a 12:12h dark: light (lights on at 6 00), with free access to food and water. This work was approved by Sultan Qaboos University (SQU) Animal Research Ethics Committee, and was conducted according to the NIH Guide for the Care and Use of Laboratory Animals, NIH publication no. 85–23, 1985, and ethical approval was also obtained from our Medical Research Ethics Committee to collect amniotic fluid (AF) from healthy women after delivery at our University Hospital.

For each gender the rats (n =30) were randomly and equally divided into five groups. Group 1 was treated with water and considered as control, group 2 was treated with human AF, group 3 was treated with goat AF, group 4 was treated with venlafaxine and group 5 was treated with venlafaxine together with human AF. A group treated with goat AF + venlafaxine was supposed to be included, but this was not possible, as there was limitation in number of rats available. AF was and given orally by gavage once day for five consecutive days at a dose of (0.2 ml/kg). Measurement of motor activity and then the FST were conducted on the sixth day.

2.2. Human AF

A sample of approximately 50 ml of AF was collected from 3 healthy women after normal delivery. Immediately after collection of the fresh samples were first filtered (No.4, 110mm diameter) and stored in a deep freezer (-80°C) for not more than 4 weeks pending utilization.

2.3. Caprine AF

About 50 ml of fresh sample was collected from 7 healthy female goats slaughtered at the slaughter House in Boshar –Muscat, Oman, and treated as above.

Motor and Behavioral tests

Locomotor Activity Test

This was always conducted between 7 00 – 10 00, as was reported before [5] using a digitalized activity meter (Ugo Basile, Comerio VA, Italy). Each rat was gently and individually placed in the activity meter cage and the vertical and horizontal movements within a period of 15 min were recorded, but the values obtained during the first five min were excluded, and only the values of the last 10 min taken.

2.4. Forced Swimming Test (FST)

The FST was carried out by the same trained observer after the motor activity test was conducted using the method described before [4]. Rat was forced to swim one at a time in a cylinder (25 cm and diameter 19 cm, containing water up to a height of 17 cm, maintained at a temperature of 25 ± 1°C. Two phases of the test were conducted. The first one was a training phase for 15 min taken. Five min were excluded, and only the values of the last 10 min.

2.5. Drugs

Venlafaxine HCl was a gift form Professor Samir Adawi (COMHS, SQU).

2.6. Statistical Analysis

Statistical analysis was carried out using GraphPad Prism 5.0 (GraphPad Software, San Diego, CA, USA). Each group consisted of at least six animals. All data are shown as means ± S.E.M. Group means were compared with an analysis of variance (ANOVA) followed by Tukey’s multiple comparison test. Values of P<0.05 were regarded as significant.

3. Results

3.1. Locomotor Activity Test

The results of this experiment are shown in Figure 1 – Figure 3. Locomotor activity test was conducted before the start of the FST. Themotor activity in male control group 282.67±13.67 was significantly lower than in the female group (316.67 ±15.4), (P<0.05). The motor activity motor in male and female rats treated with human AF were 218.2±13.6 and 211.2 ±20.1, respectively, and both were significantly lower than in the respective controls (P<0.05). The same trend was seen with goat AF, where the motor activity in male and female rats treated with goat AF were 219.5 ±16.7 and 208 ±24.5, respectively. The motor activity motor in male rats treated with goat AF was significantly higher than that in male rats treated with human AF(P<0.05). However, there was no such significant difference between male rats treated with either human or goat AF. The motor activity in the venlafaxine - treated male and female rats (276.3±14.2 and 289.5±13.4, respectively) were lower than in the control, but did not reach statistical significance. The motor activity in male and female rats treated with venlafaxine + human AF(233.67 ± 14.71and 231.34 ±14.86, respectively) were both significantly different from the control (P<0.05).

3.2. Forced Swimming Test (FST)

The results of this experiment is shown in Figure 4 – Figure 6. The immobility time in the FST in the male and female normal rats were 29.5 ±1.4 sec and 27.0 ±1.9 sec, respectively, with no significant differences between the gender (P>0.1). The immobility times in male and female rats treated with human AF (16.3 ±1.4 sec and 12.2 ±1.1 sec, respectively) were both significantly different from the respective control (P<0.05).

Also, the immobility time in male and female rats treated with goat AF (11.5 ± 1.5 sec and 14.0 ±1.0 sec, respectively) were both significantly different from the control (P< 0.05). The immobility time in male rats treated with human AF was significantly higher than that in male rats treated with goat AF (P<0.05). However, there was no such significant difference between female rats treated with either human or goat AF. The venlafaxine- treated male and female rats had immobility...
times of 25.5 ± 1.2 sec and 22.5 ± 0.8 sec (P>0.1) which was insignificant lower than that in the control. Addition of human AF to venlafaxine did not significantly affect the obtained values from either treatments.

**Figure 1.** Effect of water alone, human amniotic fluid (H-AF), goat amniotic fluid (G- AF), venlafaxine (ven) and ven+H-AF on the locomotor activity of male and female rats in the activity cage for 5 min after oral daily treatment with single dose. Each column and vertical represent the mean ± S.E.M. Different between groups are shown, and P<0.05 is considered significant.

**Figure 2.** Effect of water alone, human amniotic fluid (H-AF), goat amniotic fluid (G-AF), venlafaxine (ven) and ven + H-AF on the locomotor activity of male rats in the activity cage for 5 min after oral daily treatment with single dose. Each column and vertical represent the mean ± S.E.M. Different between groups are shown and P<0.05 is considered significant.

**Figure 3.** Effect of treatment for five days with water alone, human amniotic fluid (H-AF), goat amniotic fluid (G-AF), venlafaxine (ven) and ven + H-AF on the locomotor activity of female rats in the activity cage for 5 min after oral daily treatment with single dose. Each column and vertical represent the mean ± S.E.M. Different between groups are shown and P<0.05 is considered significant.
Figure 4. Effect of oral daily treatment for five days of male and female rats with water alone, human amniotic fluid (H-AF), goat amniotic fluid (G-AF), venlafaxine (ven) and ven + H-AF on the immobility time during 5 m in the forced swimming. Each column and vertical represent the mean ± S.E.M. Different between groups are shown and $P<0.05$ is considered significant.

Figure 5. Effect of oral daily treatment for five days of male rats with water alone, human amniotic fluid (H-AF), goat amniotic fluid (G-AF), venlafaxine (ven) and ven + H-AF on the immobility time during 5 m in the forced swimming. Each column and vertical represent the mean ± S.E.M. Different between groups are shown and $P<0.05$ is considered significant.

Figure 6. Effect of oral daily treatment for five days of female rats with water alone, human amniotic fluid (H-AF), goat amniotic fluid (G-AF), venlafaxine (ven) and ven + H-AF on the immobility time during 5 m in the forced swimming. Each column and vertical represent the mean ± S.E.M. Different between groups are shown and $P<0.05$ is considered significant.
4. Discussion

As depression is a global growing medical, economic and social problem, and the current drugs used for its management are not totally effective [13], the search for novel agents is of scientific and clinical value. Also, the study of the physiological status of the pregnant subject and the embryo and fetus particularly with regard to depression is of interest. In this experiment, we used the FST test (which is the primary and most predictive test of antidepressant drugs) to help convert the immeasurable state of depression to a measurable quantity which is called the immobility time [7,10].

The dose of venlafaxine used in this work was decided according to previous studies [18], while AF of both human and animals dose was empirical as there was no previous study of effect of AF done in the rat experimental animal model.

As shown in Figure 1 – Figure 3, the only significant decrease in immobility time was observed in goat AF (54%) vs. control, and human AF (51%) vs. control. However, the combined treatment with venlafaxine + human-AF showed an insignificant reduction (43.5%) in the immobility time compared to control group. Treatment with venlafaxine alone reduced the immobility time insignificantly by about 15.3%. This results was rather surprising and unexpected, as we used a dose that was found by others to be effective [14]. The reason for different between our findings with venlafaxine and the other studies reported positive results with this drug in the FST are not known with certainty, but may be due to the different experimental conditions or to different strain of rats used or to other factors. However, similar result with this experimental work on venlafaxine was found in one study which found that treatment with this drug had no significant effect in immobility time of FST [8].

The result of locomotor test in our experiment showed a significant decrease in rats treated with goat AF (32.5%), human AF (31.2%) and venlafaxine + human AF (25.6%), but in the group given venlafaxine only, the rats showed a slight and insignificant decrease in locomotor activity amounting to 9%. Most studies in FST are preceded by locomotor activity test because psychomotor stimulation is an important feature in the FST. In fact, the most accepted antidepressant drugs now have shown decrease in locomotor activity in the FST model [15]. However, somodamine reuptake inhibitors which is used as antidepressant drugdo increase the locomotor activity and also reduce the immobility time in FST. Earlier, Abel [1] suggested that physical activity does not account for the physiological response to forced swim testing. In conclusion, we showed in the present experiments that daily treatment of rats with human or caprine AF, showed an antidepressant-like effect in the forced swimming test, accompanied by a decrease in immobility time and locomotor activity. Our reference antidepressant drug venlafaxine, at the dose and duration used, showed insignificant effects in the FST and in motor activity test.

More research is warranted to ascertain the exact mechanism of antidepressant-like effect of AF.

References