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EFFECTS OF OESTROGEN AND/OR PROGESTERONE TREATMENT ON THE BEHAVIORAL INDICES OF ANXIETY IN OVARIECTOMIZED RATS

(With 2 Tables and 2 Figures)

By

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تأثير المعالجة بهرمون الإستروجين أو البروجيستيرون على مؤشرات السلوكيات المتعلقة بالقلق بعد إزالة المبايض فى الفئران

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اشتمل هذا البحث على دراسة السلوكيات المتعلقة فى إناث الفئران من نوع ويستر بعد إزالة المبايض. وقد تم استخدام أربعة مجموعات من إناث الفئران بعد إزالة المبايض، وحقنت المجموعة الأولى بزيت السمسم (سواغ) والمجموعة الثانية بهرمون الإستراديول والثالثة بهرمون البروجيستيرون والرابعة حقنت بكل من هرمونى البروجيستيرون والاستروجين. كما استخدمت مجموعة أخرى من إناث الفئران سليمة المبايض. وقد اثبتت هذه الدراسة أن حيوانات الفئران المستأصلة المبايض ذات مستوى معنوى مرتفع إذا ما قورنت بمثيلاتها التى لم تستأصل منها المبايض. كما أوضحت أيضاً هذه الدراسة انخفاض معنوى فى مستويات القلق لدى الإناث منزوعة المبايض بعد حقنها بهرمون البروجيستيرون فقط أو البروجيستيرون ثم أعقبه بثلاث ساعات حقن هرمون الإستروجين ولم تظهر أى اختلافات معنوية فى هذه المستويات عند مقارنتها بالإناث السليمة. كما أوضحت هذه النتائج أنه بعد حقن هرمون الإستروجين لم تحدث أى تغييرات معنوية فى مستويات القلق إذا ما قورنت بالحيوانات المنزوعة المبايض والمحقونة بالسواغ (زيت السمسم). هذا ويمكن القول بأن هرمون البروجيستيرون له تأثير مهدئ على الفئران المنزوعة المبايض وأن هذا التأثير المهدئ لا يتطلب معه وجود هرمون الإستروجين.

SUMMARY

The effects of exogenous administration of oestrogen and/or progesterone on the level of anxiety were sstudied in ovariectomized (OVX) rats. Four groups of bilaterally ovariectomized female wister rats were received subcutaneously a single dose of either 17-B-oestradiol (10 μg/kg B.W.) and/or progesterone (25 μg/kg B.W.) or sesam oil vehicle; (1 mg/kg B.W.). Another group of sham-operated female rats were used. The animals were subjected individually to the behavioral test of anxiety 3h after oestrogen or 6h after progesterone administration. Ovariectomized rats showed significantly ($P<0.05$) higher levels of anxiety than control animals. Oestrogen treatment induced no significant changes in the anxiety levels of ovariectomized rats. Progesterone decreased significantly ($P<0.05$) the anxiety state of ovariectomized rats as compared to vehicle-injected OVX- animals. These results suggest that progesterone have an anxiolytic effect on ovariectomized rats and such effect did not require the presence of oestrogen.

Key words: *Oestrogen, Progesterone, Bchavior, Anxiety, Rats.*

INTRODUCTION

A growing body of evidence has demonstrated that ovarian hormones can modify the spontanous behavior (anxiety) that can be measured in the elevated plus-maze apparatus (Diaz-Veliz *et al.*, 1994; 1997).Ovariectomy (ovx) was found to enhance avoidance conditioning, whereas systemic administration of oestrogen was seen to reduce this behavior However, progesterone has been shown to prevent the impairment of the active avoidance acquisition at oestrus and could antagonize the depressant effects of oestrogen (Diaz-Veliz *et al.*, 1991; 1994). Furthermore, Mora *et al.* (1996) reported that ovariectomized rats exhibit higher levels of anxiety than intact female rats when assessed either by elevated plus-maze or by retention of passive avoidance conditioning tests. Further support of the relationship between ovarian hormones and behavioral indices of anxiety was reported by Madeha *et al.* (2002) who demonstrated that ovariectomized adult female rats displayed higher levels of anxiety than intact sham-operated animals. These results led us to suggest that the increased anxiety levels in ovariectomized rats are possibly due to the eleminated anti-anxiety effect of the ovarian hormones (oestrogen and/or progesterone). Other experimental evidence has shown that ovarian hormones exert anti-

anxiety effect in paradigms that typically used to measure the anxiolytic potency of diazepan and serotonin (FernandeGuasti and Picaazo, 1990; Nomikos and Spyraki, 1988).

The present studies was performed in order to determine the influence of ovarian hormones replacement on the anxiety levels of ovariectomized adult female rats. The ovariectomized animals were treated with oestradiol and/or progesterone and then submitted to measure anxiety levels in the elevated plus-maze.

MATERIALS and METHODS

Experimental animals:

Female wister rats weighing 200-220 g, were used in the present study. Animals were maintained under standard laboratory conditions: room temperature (22 ±2 C) was kept constant, 12 : 12 h light-dark cycle was employed with light on at 6:00 a.m. Standard laboratory food pellets and tap water were available ad *libitum*. All experimental procedures conformed to the laws on animal welfare. None of the rats were previously subjected to any experimental procedure.

A group of 32 rats were bilaterally ovariectomized under ether anaethesia. After 30 days of surgical operaation they were assigned to 4 groups (8 rats/group) according to the hormonal treatments. Another group of female rats (N=8) was sham-operated and used as controls for surgical operation.

Surgical Procedures:

Ovariectomy was performed under ether anaesthesia and the ovaries were removed via a dorsolateral approach. Sham-operated (intact) female rats were similarly anaesthetized and received a surgical incision, but the ovaries were not removed.

Hormonal Treatments:

Ovariectomized rats were injected subcutaneously with a single dose of 17 ß- oestradiol and/ or progesterone dissolved in sterile sesame oil vehicle.The injection started 30 days after surgery. The first group of ovariectomized rats was received a single (acute treatment) dose of oestradiol at concentration of 10 µg/kg body weight in sterile sesame oil injected subcutaneously (s.c.) and then tested for their anxiety levels after 3 hours following injection. The second group was injected (s.c.) with a single dose of progesterone at a concentration of 25 µg/kg body weight dissolved in sterile sesame oil and left for 6 hours before tested

for anxiety.The third group of rats was injected (s.c.) with a single dose of progesterone (25 μ /kg. B.W.), then 3 hr later, these rats were received another single dose of 17-B-oestradiol (10 μ/kg.B.W.). The behavioral test began 6 hr after progesterone injection, i.e. 3 hr after oestrogen treatment. These particular time coarses for measuring the anxiety levels after injections with oestradiol (3h) and progesterone (6h) were chosen becaause the primary (genomic) effect of these hormones occur shortly after injections and to avoid any other behavioral changes that might appear later on due to the sensitivity of brain transmitters toward these ovarian hormones (Segal and Koide, 1979; Nomikos aand Spyraki, 1988). The fourth group of ovariectomized rats was injected (s.c.) with sterile sesame oil (1 ml/kg.B.W.) and considered as control group for hormonal treated ovariectomized rats. Both hormones were dissolved in sterile sesame oil and injected subcutaneously in the dorsal region of the neck. The dose of either oestradiol or progesterone that injected to the ovariectomized rats were chosen according to the published reports of Diaz-Veliz *et al.* (1991, 1994).

Plus-maze apparatus:

The elevated plus-maze test has been in use as a rodent model of anxiety for a decade, and was representative of those tests that are based upon the study of spontaneous behavior patterns and which have high ethological validity (Dawson and Tricklebank, 1995; Rodgers and Dalvi, 1997). The elevated plus-maze test was the most popular of all currently available animal models of anxiety, and affords an excellent example of a model based on the study of unconditioned, or spontaneous behavior (File, 1992; Handley and McBlane, 1993; Rodgers and Cole, 1994).

The method of measuring the anxiety levels in rats was published elsewhere (Madeha D., 2000). Briefly, a video camera was mounted vertically above the maze, and the behaviour was scored by means of a monitor and computer keyboard in an adjacent room. Each rat was placed in the central square facing the closed arm, and was allowed to explore freely the maze for 5 min under bright light-illumination. At the end of each trial, the maze was wiped clean with a damp cloth, to remove excreta and individual odours that might have affected the behavior of the animal tested subsequently. The times spent in the open and closed arms were computed. The criterion for arm entry was '4 paws in one of the arms', while the criterion for exit was '2 paws out of the arm'. In addition, the percentage of time spent in the open arms was calculated [open time : (open time + closed time) x 100]. By convention,

an increase in the percentage of time spent in the open arms was interpreted as an anxiolytic response, whereas the number of entries into closed arms was taken as a measure of general activity, (e.g. anxiogenic response).

Statistical analysis:

In all illustrations of the data, mean ± standard error of the mean (SEM) were depicted. Statistical analysis was based on raw data, and performed using a software package (SigmaStat; Jandell Scientific). Data were subjected to one-way analysis of variance (ANOVA), followed by parametric or non-parametric pairwise comparisons, depending on whether the data passed a normality test or not. The level of significance in all tests was preset at $p \leq 0.05$.

RESULTS

The analysis of ovariectomized rats behavior in the elevated plus-maze (Table 1,2 and Figures 1,2) revealed the influence of the ovarian hormones on the percentage of time spent in the open arms and latencies to first entry into open arms. Ovariectomized rats injected with vehicle (sesame oil) showed significant decrease in the percentage of time spent in the open arms (Fig. 1) and significant increase in the latencies to first entry into open arms (Fig. 2), as compared to sham-operated (intact) females. However, ovariectomized rats injected with a single dose of progesterone showed no significant differences in the time spent into open arms (Fig. 1) and latencies to first entry into open arms when compared with intact adult female rats. This response indicate a significant ($P<0.05$) effect of progesterone on normalizing the plus-maze parameters in ovariectomized rats. Treatment with progesterone and oestrogen together in ovariectomized rats showed no significant differencies in the percentage of time spent in the open arms and latencies to first entry when compared with either sham-operated rats or with those treated with progesterone alone (Figs 1 and 2) showed that a single dose of oestrogen to ovariectomized rats did not significantly affect the percentage of time spent in open arms or latencies to first entry as compared to those measured in ovariectomized rats injected with vehicle.

In the plus-maze, anxiety is reflected to the percentage of time spent in the open arms and latencies to first entry into open arms. These two parameters were interpreted as follows; increase in percentage of

time spent in open arms and decrease in latencies reflect reduced anxiety (anxiogenic effect) and vice versa (anxiolytic effect). These results showed that ovariectomy in rats induced significant increase in anxiety levels as compared to sham-operated animals, and this response was inhibited significantly by treatment with progesterone but not with oestrogen. There were no signoficant differences in the anxiety levels between ovariectomized rats injected with vehicle and those injected with a single dose of oestrogen. These results also indicated that the anxxiolytic effect of progesterone in ovariectomized rats treated with progesterone alone, did not require the presence of oestrogen as shown in rats treated with progesterone followed by oestrogen.

DISCUSSION

The results of the present work clearly showed that treatment with physiological progesterone, rather than oestrogen inhibited the increase in anxiety levels of ovariectomized rats. To this extent, the anxiolytic effect of progesterone did not require the presence of oestrogen. These results also suggest that treatment with a single dose of oestrogen either alone or in combination with progestrone did not suppress the incresed anxiety of ovariectomized rats at least at the concentration of the injected dose.

Several reports have shown that oestrus cycle and ovarian hormones (oestrogen and progesterone)could modify the spontaneous behavior that was exhibited in the plus-maze apparatus (Diaz-Veliz *et al.*, 1989; 1997). Furthermore, ovariectomy was found to increase anxiety levels as compared to intact (sham-operated) females (Mora *et al.*, 1996; Bitran and Dowd, 1996; Madeha D., 1998; Madeha D., etal., 2002). In addition, it has been reported that treatment with progesterone induced anti-anxiety (anxiolytic) effect in the ovariectomized rats (Mora *et al.*, 1996). Moreover, progesterone-derived neurosteroid (tetrahydropregesterone, THP) has been shown to induce anxiolytic effect in ovariectomized stressed female rats (Lambert *et al.*, 1995; Madeha D., 1998).

In view of the above, the results of the present work provide further support for the relationship between ovarian hormones and the behavioral indices of anxiety as measured in the elevated plus-maze apparatus.

In our experimental condition (plus-maze exploration under bright light illumination), treatment with single physiological dose of oestrogen failed to reduce the behavioral indices of anxiety in ovariectomized rats. These results were consistent with Mora *et al.*, 1996 who failed to demonstrated significant changes in plus-maze exploration in ovariectomized rats after treatment with oestrogen. Moreover, several studies have demonstrated that treatment with single dose of oestrogen into ovariectomized rats antagonize the anxiolytic properties of diazepan (Nomikos and spyraki, 1988) or the anxiolytic effect of progesterone (Mora *et al.*, 1996). Furthermore, the oppsite effects of oestrogen and progesterone on avoidance conditioned response were attributed to the anxiogenic effect of oestrogen and the anxiolytic effects of prrogesterone (Diaz-Veliz *et al.*, 1994).

Several lines of evidence explained the anxiolytic effects of progesterone or its naturally produced metabolites. The ring A-reduced metabolite of progesterone (tetrahydroprogesterone, THP) has a positive and powerful anxiolytic actions in rats (Lan and Gee, 1994; Lambert *et al.*, 1995). Gama-amino-buteric acid (GABA) is the principle inhibitory neurotransmitter that involved in the regulation of anxiety in the central nervous system (Costa, 1980; Gray and Books, 1984; Lambert *et al.*, 1995). There were studies suggesting that progesterone and its naturally produced metabolities act directly to stimulate release of GABA and incresed number of GABA receptor (McEwen, 1991; 1992; Baulien, 1992; Windle *et al.*, 1997). These action occur at concentrations that were physiologically and they were rapid in onset (Bitran *et al.*, 1991; McEwen, 1991)..

On the basis of the above, the present results suggest that progesterone was the gonadal steroid that exhibit and exert the major anxiolytic effect in ovariectomized female female rats.

In conclussion, the results of the present work provide a further support for the role of ovarian hormones in modulation the behavioral indices of anxiety in female rats. Injection of single dose of progesterone induced significant decrease in anxiety of ovariectomized rats. The anxiolytic effect of progessterone did not require the presence of oestrogen. Injection of oestrogen failed to reduce the anxiety levels in ovariectomized rats.

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LEGENDS

Fig. 1: Showing the percentage of time spent into open arms of the plus-maze for sham-operated female rats (Int); ovariectomized (OVX) rats injected with seame oil (Oil) ; ovariectomized rats treaated 17-B-oestradiol (OVX+E2); ovariectomized rats treated with progesterone (OVX+P) and ovariectomized rats treated with oestradiol followed by progesterone (OVX+E2+P). Astric indicate statistically significant (P> 0.05) differences when compared to OVX +OIL.

Fig 2: Showing latencies to first entry into open arms of the plus-maze for for sham-operated female rats (Int); ovariectomized (OVX) rats injected with seame oil (Oil); ovariectomized rats treaated 17-B-oestradiol (OVX+E2); ovariectomized rats treated with progesterone (OVX+P) and ovariectomized rats treated with oestradiol followed by progesterone (OVX+E2+P). Astric indicate statistically significant (P> 0.05) differences when compared to OVX +OIL.

Table (1): Time in open arms/Total time in maze (%).

	Sham-Opers	OVX + Oil	OVX + E2	OVX + P	OVX + E2 + P
Mean + S.E.	*61.7 ±1.5	41.7 ± 1.2	41.8 ± 1.4	*63.1 ± 2.5	*58.4 ± 2.9

Table (2): Latency to first into open arms (Seconds).

	Sham-Opers	OVX + Oil	OVX + E2	OVX + P	OVX + E2 + P
Mean + S.E.	*17.9 ± 0.6	32.4 ± 1.3	32.1 ± 0.8	*16.3 ± 0.8	*19.3 ± 1.3

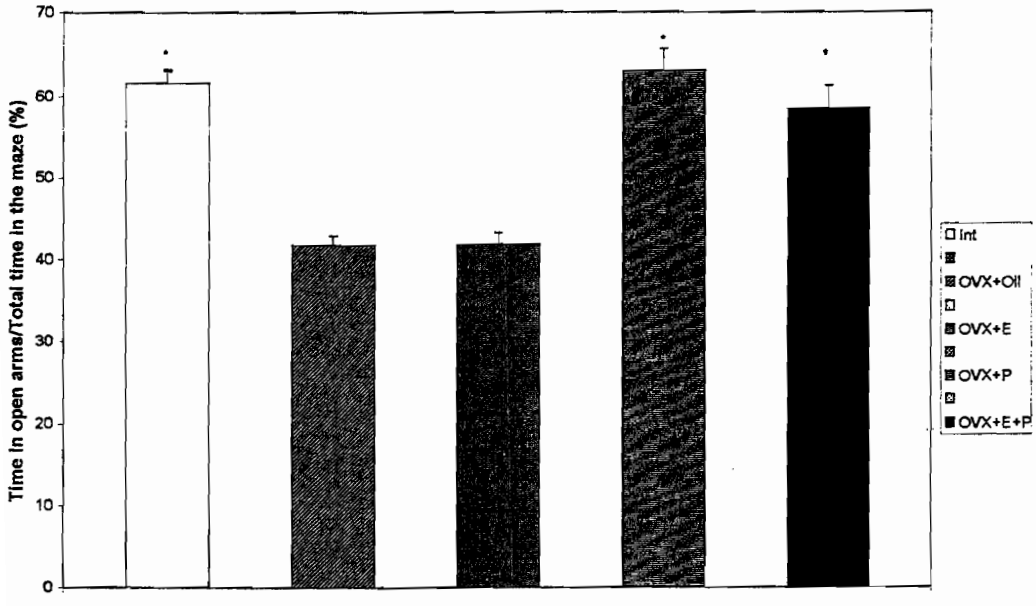
Time in open arms/Total time in the maze (%)
70
60
50
40
30
20
10
0
Int
OVX+Oil
OVX+E
OVX+P
OVX+E+P

Fig. (1)

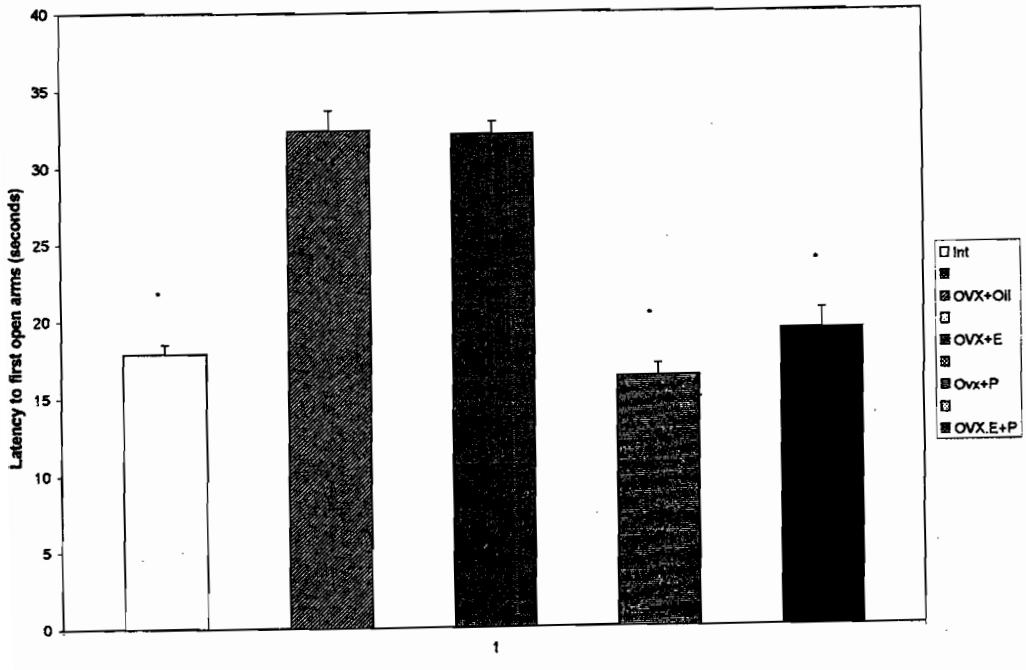
Latency to first open arms (seconds)
40
35
30
25
20
15
10
5
0
1
Int
OVX+Oil
OVX+E
Ovx+P
OVX.E+P

Fig. (2)