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A Possible Role for Oxytocin in the Response to a Psychological Stressor

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MUIR, J. L., R. BROWN AND H. P. PFISTER. *A possible role for oxytocin in the response to a psychological stressor.* PHARMACOL BIOCHEM BEHAV **25**(1) 107–110, 1986.—Recent evidence suggests that oxytocin (OXT) potentiates corticotropin releasing factor-induced secretion of ACTH. The present study was therefore designed to investigate the possible role of oxytocin in the response to predictable and unpredictable novelty stress. The results clearly demonstrate that oxytocin produced a significant increase in corticosterone in all OXT treated animals. Repeated unpredictable exposure also produced a more substantial increase in corticosterone than predictable exposure to the same stressor. However, a significant interaction between stress and oxytocin was not obtained. It was concluded that whereas corticosterone is released in response to most types of stress, administration of oxytocin does not potentiate the corticosterone response to psychological stress.

Oxytocin Corticosterone Novelty Predictable stress Unpredictable stress

ACTIVATION of the hypothalamic-pituitary-adrenocortical axis is known to occur under conditions of stress, and appears to be particularly responsive to psychological rather than physical stimulation [18]. For example, a psychological stressor such as novelty produces a rapid rise of 11-hydroxycorticosterone (11-OHCS) output from the adrenal cortex in the rat [6, 14, 23]. This form of stress is also capable of producing elevations in plasma corticosterone comparable to those following the initial exposure to a more intense stimulus such as electric shock [4, 7, 13]. Furthermore, as the rat reaches some degree of familiarity with the novel environment, a corresponding reduction in 11-OHCS has been observed [21–23]. Pfister [21] reports that rats habituate to a novel environment after 5 days of repeated (30 minute daily) exposures.

It is well known that the activity of the adrenal cortex is regulated by the level of adrenocorticotropic hormone (ACTH) in blood plasma and that ACTH secretion is primarily mediated by the hypothalamic hormone 41-residue corticotropin-releasing hormone (CRF) [27]. However, there is a growing body of evidence that additional hormones, such as vasopressin and oxytocin, may also be involved in the regulation of ACTH secretion [1, 8, 10].

Oxytocin (OXT), a nonapeptide hormone secreted by the neurohypophysis, has traditionally been regarded as a reproductive hormone. Its main reproductive effects are the contraction of the uterus during parturition and contraction of the myoepithelial cells of the mammary glands, resulting in the ejection of milk [11,26]. Recently, it has been reported that a substantial amount of OXT is secreted in response to physical stress [8, 16, 28]. Although little is known as to the role of OXT in the stress experience, it appears that OXT may contribute to the regulation of ACTH release by potentiating CRF-induced ACTH secretion [1, 5, 10]. This is supported by the observation that OXT is present in hypophysial portal blood at concentrations that can potentiate the ability of CRF to release ACTH in vitro [1,9].

However, the release of OXT does not occur in all stress situations but instead appears to be a selective response. While large increases in secretion have been reported following restraint, forced swim and ether stress [8, 16, 28], OXT levels remained unchanged following cold exposure [8,16]. This occurred despite the fact that ACTH levels were substantially increased in the animals receiving cold exposure [8]. In addition, it has been observed that while immobilization produces a distinct decrease of oxytocin in the hypothalamus and the neurohypophysis, such an effect does not occur in rats exposed to cold [12]. It appears that although activation of the hypothalamic-pituitary-adreno-cortical axis is a constant feature of most types of stress, the hypothalamic and neurohypophysial contribution to the control of ACTH may be different in response to different types of stress.

More recently, King, Brown and Kusnecov [15] have investigated the effect of OXT on the startle response. A significant increase in this response was observed in those animals administered 5.8 or 11.6 IU OXT/kg compared to animals administered a vehicle solution. According to King *et al.* [15], these data suggest an increase in fear/emotionality and/or reflex reactivity following administration of OXT. Plasma 11-OHCS levels suggested increased fear/emotionality in all groups, including the control group. The failure to obtain larger increases in 11-OHCS output by OXT treated animals may be explained by a 'ceiling' effect being reached

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in the 11-OHCS output to this stress situation. It may therefore be necessary to vary the severity of the stressor in order to overcome such a problem. One way in which this has been done is by manipulating the pattern of exposure to the stressor.

Mason [18], in a review of psychoendocrine research, concluded that situations involving anticipation or unpredictability of a previously experienced stressor produced a greater corticosterone steroid elevation than the stressor itself. Experiments carried out using predictable and unpredictable shock [2,3] have found that animals prefer predictable exposure to the shock. Further support for the greater aversiveness of an irregularly applied stress is provided by information on the corticosterone levels of animals following restraint stress [24]. In this study, the initial rise in corticosterone following restraint stress abated when the stress procedure was presented chronically in a regular or predictable manner. However, no adaptation occurred when animals were restrained for a similar period of time in an unpredictable manner.

The present experiment was therefore designed to investigate the role of OXT in the stress response. More specifically, this study examined the possible role of OXT in the response to a psychological stressor (novelty). Since the severity of the stressor may be an important factor when investigating 11-OHCS output, the predictable/unpredictable paradigm was included.

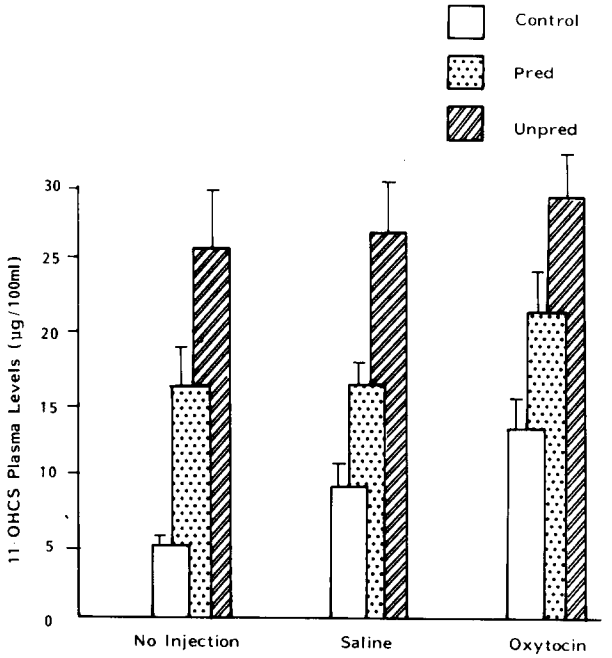

FIG. 1. Steroid response of CTRL, PRED and UNPRED animals pretreated with oxytocin or saline injections, or no injections.

METHOD

Animals

Seventy-two nulliparous female Wistar rats 90–100 days old at the start of the experiment were used. Three weeks prior to testing, all animals were individually housed in a fully air-conditioned holding room at 22±1°C. A 12:12 light/dark cycle was instituted. Food and water were provided ad lib.

Glucocorticoid Assay

The glucocorticoid stress response measured was the plasma level of free 11-OHCS, the predominant glucocorticoid secreted in the rat [17,20]. At the times indicated in the procedure, animals were sacrificed by decapitation. The blood was collected in heparinized tubes and centrifuged to obtain cell-free plasma which was then frozen. Corticosterone levels in plasma were obtained subsequently by the fluorometric method of Mattingly [19] which is specific for free plasma 11-OHCS.

Apparatus

The novelty cage was made of 1 cm wire mesh of 2 mm thick wire with a hinged lid and external dimensions 14.5×20×26 cm. The novelty cage was placed inside the rat holding room described above.

Procedure

The animals were randomly allocated to three groups of 24 rats each: a control group (CTRL), a predictable stress group (PRED) and an unpredictable stress group (UNPRED). Each of these groups were further subdivided into three treatment groups (n=8): a group which received no injections (NI), a group which received 1 ml/kg injections of the vehicle solution (0.9% saline) (VS) and a group which received injections of 5.8 IU OXT/kg (OXT) (see Table 1). All injections were given via the IP route.

TABLE 1

ALLOCATION OF 72 NULLIPAROUS RATS TO 9 GROUPS OF 8 ANIMALS PER GROUP

Oxytocin	No Injections	Vehicle	Solution
CONTROL	CTRL/NI	CTRL/VS	CTRL/OXT
PRED	PRED/NI	PRED/VS	PRED/OXT
UNPRED	UNPRED/NI	UNPRED/VS	UNPRED/OXT

The dose of oxytocin was chosen for two reasons. Firstly, King *et al.* [15] have shown that 5.8 IU OXT/kg is more effective in potentiating the startle response of rats to auditory stimulation than two larger doses of oxytocin (11.6 IU OXT/kg and 23.2 IU OXT/kg). Secondly, Rivier and Vale [25] have shown that doses of 8–24 nmoles OXT/kg are effective in elevating ACTH secretion in freely moving rats. Our dose of 5.8 IU OXT/kg equates to approximately 12–14 nmoles OXT.

Animals of the three CTRL groups were used for determination of plasma 11-OHCS base levels. Other than drug treatment (CTRL/VS and CTRL/OXT), all control animals were left undisturbed until decapitation and assay. Animals allocated to the three PRED groups were subjected daily, for five successive days, to a single 30 min exposure to the novel apparatus. For these groups, novelty treatment began each day 2 hr prior to light onset, and thus at the trough of the circadian rhythm in relation to 11-OHCS levels [23]. Injections for PRED/VS and PRED/OXT animals were given immediately prior to each exposure to the novel apparatus. At

this time (i.e., 2 hr prior to light onset) injections were also given to animals of the CTRL/VS and CTRL/OXT groups.

Those animals allocated to the UNPRED condition received four exposures to the novel apparatus on a random schedule (one 30 min exposure to the novel apparatus each day at a randomly selected time within each 24 hr period). However, on the fifth day, novelty treatment for UNPRED animals began 2 hr prior to light onset and therefore at the trough of the circadian rhythm. Once again injections for animals of the UNPRED/VS and UNPRED/OXT groups were administered immediately prior to each exposure to the novel apparatus.

Novelty treatment for animals of the PRED and UNPRED groups was administered by picking up each rat by the base of the tail and gently placing it in the novel apparatus. Thirty minutes later, the rat was again picked up by the base of the tail and returned to its holding box. After their final manipulation, animals of the PRED and UNPRED groups were removed in their novelty cage from the holding room and taken to the preparation room. Blood plasma was collected within 60 sec of removal from the holding room. Similarly, at this time (2 hr before light onset), the undisturbed control animals (CTRL) were removed in their boxes to the preparation room where they were sacrificed.

RESULTS

The changes in plasma 11-OHCS levels as a function of OXT and stress treatment are shown in Fig. 1. Two way analysis of variance revealed significant OXT effect, $F(2,63)=3.38$, $p<0.05$, and a significant stress treatment effect, $F(2,63)=34.0$, $p<0.001$. The OXT $\times$ stress treatment interaction failed to reach significance.

Scheffe post-hoc comparisons on the OXT effect showed that the OXT injected animals had significantly higher plasma 11-OHCS levels than both, animals which received no injections ($p<0.001$) and those which were saline injected ($p<0.05$). No significant change was observed in the plasma 11-OHCS levels of animals which received saline injections compared to animals which received no injections. Scheffe post-hoc analysis of the stress treatment effect revealed a significant increase in 11-OHCS levels between CTRL animals and animals of the PRED stress group ($p<0.001$) and between CRTL animals and animals of the UNPRED stress group ($p<0.001$). There was also a significant increase in 11-OHCS level of the UNPRED group as compared to the PRED group ($p<0.001$).

DISCUSSION

The results of this experiment clearly demonstrate that administration of 5.8 IU/kg OXT produced a significant increase in plasma 11-OHCS levels of animals of the CTRL/OXT, PRED/OXT and UNPRED/OXT groups. Support for the greater aversiveness of an irregularly applied stress was also provided by this study. Plasma 11-OHCS levels of animals exposed to the novelty stressor on an unpredictable schedule were substantially larger than those of animals receiving predictable exposure to the same stressor. This finding is in agreement with the results obtained by Quirce *et al.* [24] using predictable and unpredictable restraint stress.

There was no significant interaction between OXT and stress treatment, as suggested by the finding that OXT potentiates CRF-induced release of ACTH secretion [1,5]. However, inspection of Fig. 1 suggests a general upward trend in 11-OHCS levels between the no injection and saline injected groups and between the saline and OXT groups. Furthermore, it appears that this upward trend may not be equally shared by each stress treatment group. For example, while the UNPRED groups display similar high levels of 11-OHCS, (25.7, 26.8 and 29.2 μg/100 ml plasma for NI, VS and OXT groups respectively), our CTRL groups show a marked difference (5.13, 9.38 and 13.26 μg/100 ml for NI, VS and OXT groups respectively).

King *et al.* [15] have shown that while injection of varying doses of oxytocin produced an observable behavioral difference as measured by an auditory startle response, this difference was not apparent when examining plasma 11-OHCS levels. These plasma levels [15] suggested that each animal was equally stressed regardless of whether the animal received oxytocin or saline. Our present results show a levelling effect of 11-OHCS concentration at our highest level of stress. It would therefore seem that the injection of oxytocin at base levels does not significantly potentiate the corticosterone response to novelty stress.

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