Confirmation of the inhibitory influence of exogenous oxytocin on cortisol and ACTH in man: Evidence of reproducibility

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Abstract. The influence of low-dose oxytocin perfusion (32 mIU/min) on ACTH and cortisol plasma levels was tested in 8 normal male volunteers (age 18–26). The 1-h oxytocin perfusion periods were preceded and followed by two 1-h saline control periods. During the first period, there was a slight ACTH concentration decrease in 4 individuals. Oxytocin perfusion induced a clear-cut plasma ACTH decrease in 7 out of the volunteers, and a slight decrease in the remaining one. During the second saline infusion, there was a marked ACTH increase in 7 out of the volunteers, and a weak increase in one (P < 0.0001). A similar pattern was observed in the plasma cortisol levels (P < 0.00001). Furthermore, a control saline perfusion was performed in 4 of the 8 volunteers and compared to the 4 corresponding oxytocin perfusion tests: the differences between the 2 sets of tests was highly significant both for ACTH (P < 0.003) and for cortisol (P < 0.007). Lastly, the reproducibility of this inhibitory influence was retested in 4 volunteers: the tests were repeated under the same conditions 7 days later. There were no global differences between the 2 sets of tests either for ACTH (NS) or for cortisol (NS). ACTH and cortisol concentration fluctuations during the period between each set of tests were not significant. The following variations were measured for ACTH (NS) and for cortisol (NS). The present results confirm the inhibitory influence of low-dose oxytocin perfusion on ACTH and cortisol levels in normal males. Moreover, we demonstrate an increase at the end of the oxytocin perfusion (second control saline infusion). The reproducibility of the inhibitory effect at a one-week interval in 4 volunteers brings new argument against a non-specific stress influence on corticotroph function.

In previous works we demonstrated that in normal humans exogenous oxytocin (OT) induces a decrease in basal cortisol levels (Chiodera & Legros 1981), basal ACTH (Legros et al. 1984), as well as stimulated ACTH and cortisol (Legros et al. 1982). By using a slightly modified experimental protocol, Lewis & Sherman (1985) recently did not confirm such an inhibitory effect and hypothesized that part of our experimental results could be due to a non-specific stress influence during the early part of the infusion period.

In the present work we tested the influence of low-dose (32 mIU/min) OT perfusion after a 1-h saline control period and continued the saline infusion for 60 min beyond the end of the OT infusion period. This rigorous methodology was designed to eliminate the influence of any non-specific stress action in the 8 volunteers. Furthermore, blood neurophysins were measured as an index of the putative influence of exogenous OT on endogenous neurophysitary function. In 4 individuals we also repeated the test series after 7 days in order to evaluate the reproducibility of the response.
Material and Methods

Oxytocin inhibitory tests were carried out in 8 normal male volunteers selected from members of the medical staff (age 18–26). None of them exceeded the ideal body weight by more than 20% (Body Mass Index: 19.9–23.8).

In each fasting recumbent individual, catheters were placed in a forearm antecubital vein in both arms at 09.00 h and from 09.30 h to 10.30 h, they received a saline infusion (100 ml of 9% NaCl); from 10.30 h to 11.30 h, a similar amount of saline containing synthetic OT (Syntocinon®, Sandoz, Basel) at the rate of 32 mIU OT/min, was infused. From 11.30 h to 12.30 h, the infusion was continued, but with 100 ml of saline alone. Blood samples were drawn from the opposite catheter every 15 min from 09.30 h to 12.30 h. Similar tests were performed under the same conditions 7 days later in 4 volunteers. Control saline tests were also carried out in these 4 volunteers: identical manipulations were performed hourly except that synthetic OT was replaced by a similar amount of saline.

The ACTH concentration was assayed on plasma samples using a CEA-IRE-SORIN kit (normal values < 20 to 100 ng/l, intra-assay variability 9%). Cortisol was assayed using the Biodata® kit (Milan, Italy, normal values 250–550 nmol/l, intra-assay variability 4.5%). Arginine-vasopressin (AVP)-neurophysin (hNpI) and oxytocin-neurophysin (hNpII) were measured in the 8 OT perfusion tests by RIA using the system of Dax et al. (1979) (hNpI: normal values: 440 ± 20 ng/l, intra-assay variability 6.1%, inter-assay variability 7%)), hNpII: normal values: 1350 ± 150 ng/l, intra-assay variability 3.3%, inter-assay variability 5.7%).

Statistical analyses were performed using ANOVA tests to compare the 3 periods in the 8 tests and sequence influence within the 4 repeated tests. Oxytocin perfusion influence was analysed using two-way variance analysis for successive repeated measures: the two main factors were the periods (saline-OT-saline) and the time during each of these periods. In the case of non-equality of variances, the degrees of freedom were decreased by the Huynh method (Huynh & Feldt 1976). In the reproducibility study, the same statistical method was used, but the primary major factor was the test sequence.

![Graph](image_url)

**Fig. 1.**

Individual plasma levels of ACTH (top) and cortisol (bottom) during a first control saline infusion, followed by an OT infusion (32 mIU/min), followed by a second control saline infusion in 8 normal male volunteers.
Fig. 2.

Individual plasma levels of ACTH (top) and cortisol (bottom) during a first control saline infusion, followed by an OT infusion (32 mIU/min), followed by a second control saline infusion in 4 normal male volunteers. The charts on the left and the middle represent the same 4 volunteers in the same set of tests, but with a one-week interval between test sets. The chart on the right represents the same 4 volunteers in the same set of tests with saline only.

Results

The individual results of plasma ACTH and cortisol levels in the 8 tests are shown in Fig. 1. During the control period there was a slight ACTH decrease in 4 individuals. Infusion of OT induced a clear-cut decrease in plasma ACTH concentration in 7 or the 8 volunteers, and a slight decrease was observed in the remaining one. During the last saline infusion the ACTH level increase was sharply marked in 7 of the volunteers, whereas it was weak in the one volunteer whose levels had varied least during OT perfusion. A similar pattern, although less marked, was observed for plasma cortisol: the volunteer (x on the graph) with the smallest ACTH inhibition also showed the smallest cortisol concentration variation. There was a significant difference between the 3 periods for ACTH $F^2_{34} = 72.88, P < 0.00001$). The interaction between the periods and time was also very significant for ACTH $F^4_{42} = 21.11, P < 0.00001$) and for cortisol $F^4_{42} = 44.31, P < 0.00001$).

Basal hNpI (600 ± 100 ng/l) and hNpII (1500 ± 500 ng/l) were within the normal range. Blood levels were stable during the first $F^2_{41} = 1.4$ and 0.5, respectively, (NS) and the second $F^2_{41} = 0.2$ and 0.5 (NS) saline infusion. During OT infusion, there was a slight but significant fluctuation of hNpI $F^2_{41} = 6.6, P < 0.003$) and hNpII $F^2_{41} = 2.6, P < 0.08$). There was, however, no significant increase or decrease in the blood neurophysin concentration during the infusion period.

Individual results in the 4 OT perfusion tests at a one-week interval and the 4 control saline tests are shown in Fig. 2. There were no global differences between the 2 OT-perfusion sets of tests for ACTH $F^1 = 0.57, P: NS$) or for cortisol $F^1 = 0.03, P: NS$). Moreover the fluctuations during the period between both sets of tests did not differ for ACTH $F^2_{48} = 2.58, P: NS$) or for cortisol $F^2_{36} = 1.9, P: NS$).
Comparing the control saline infusion with each of the OT perfusion yielded highly significant global differences both for ACTH $F_{12}^3 = 4.43$, $P < 0.003$ and for cortisol $F_{12}^3 = 13.00$, $P < 0.007$.

When comparing the 3 periods of each test (saline-OTT-saline) or (saline/saline-saline) there was no difference for the first saline infusion (ACTH, $F_3 = 0.78$, $P$, NS; cortisol $F_3 = 1.93$, $P = 0.20$, NS), in contrast to for the sequence (OTT or saline) (ACTH $F_{12}^3 = 6.22$, $P < 0.006$; cortisol $F_{12}^3 = 18.30$, $P < 0.00001$) and the third sequence (saline after OT, saline after saline) (ACTH $F_3 = 38.77$, $P < 0.00001$; cortisol $F_3 = 7.91$, $P < 0.007$).

Discussion

In the present work we confirm the inhibitory influence of low-dose exogenous OT on ACTH and cortisol plasma levels in normal male volunteers.

Furthermore, our methodology was devised to counter the argument that this inhibitory effect could be an artifact caused by a non-specific stress at the beginning of the test. It is apparent that the decrease observed during the OT infusion was much more marked than the slight, or no decrease observed during the first saline period. On top of that, at the end of the OT infusion, there was a marked increase in ACTH and cortisol levels while the saline perfusion was continued; such a secondary increase is not observed in the 4 control tests. A non-specific effect owing to perfusion removal then is very unlikely. Lastly, a second series of tests performed after a one-week interval in 4 volunteers produced very similar results, demonstrating that this inhibitory action is reproducible in each individual.

The inhibitory action could be of physiological significance, as it has been shown that in the primate, neurogenic stress induces a decrease of OT release (Gibbs 1984) which is time-related to an inverse fluctuation of plasma cortisol. This inverse relationship between OT and cortisol has also been demonstrated during dexamethasone treatment (Kalin et al. 1985).

The discrepancy between our results and those recently published by Lewis & Sherman (1985) must be discussed. These authors studied only 4 individuals, whereas we studied at least 6 in each experiment (Legros et al. 1982, 1984). Also, the first two experiments by Lewis & Sherman were carried out in the early morning (06.30 h and 03.00 h), whereas all our experiments took place after 09.30 h, a period where spontaneous cortisol fluctuations are minimal (Weitzman et al. 1971). Since it is known that there is marked circadian fluctuation in central OT release (Sakar & Gibbs 1984; Reppert et al. 1984), it is conceivable that the sensitivity to exogenous OT may vary according to the nycthemer. Moreover, although Lewis & Sherman stated that the OT used in their studies was biologically active, they did not check the exact peripheral plasma OT concentration achieved by RIA as we did during our previous perfusion study. OT instability at physiological pH and fixation on glass vials is not uncommon. Therefore it is of great importance to check the precise amount of peptide injected. In their first experiment, Lewis & Sherman used high doses (90 and 120 mIU/kg/120 sec) in a bolus injection without any detectable side effects, whereas in our hands, such high doses led to severe side effects impairing the interpretation of results (unpublished observations). Lastly, in their studies, the 4 volunteers were admitted to the Clinical Research Centre at 21.00 h on the day prior to the experiment, whereas in our studies volunteers were admitted at 08.00 h on the day of the test. Although one could postulate that bed rest during the night prior to the experiment gave a better ‘resting’ state for the test, sleeping one single night in unfamiliar surroundings can be troublesome (first night effect) and could presumably modify corticotroph responses.

The way OT acts on the ACTH release is still unknown and was discussed previously (Legros et al. 1984). Gaillard et al. (1984) showed that the affinity of OT for AVP rat anterior pituitary receptors was only 1.1% that of AVP suggesting that a competition at that level, possibly could be observed for very high OT/AVP ratios. Such a weak affinity of OT for AVP anterior pituitary receptors was recently confirmed by Koch & Lutz-Bucher (1985). An action of exogenous OT on endogenous AVP or OT release also would seem less likely since, in the present work, there was no systematic modification of hNpI and/or hNpII in the course of the oxytocin infusion. The increased fluctuation of both neurophysins during the perfusion indicates, however, that some kind of ‘dysregulation’ of the endogenous neuropituitary release could occur. A positive feed-back of OT on
its own secretion has been described previously in the rat (Moos et al. 1984). To our knowledge, the influence of OT on AVP release has not been studied before.

A supra hypothalamic action of OT could be postulated too, since OT receptors are present in the limbic system (hippocampus) and since neurophysiological studies indicate an inhibitory action of OT at this level (Mühlenthaler et al. 1984).

It is also of interest to note that OT can be inhibitory for the release of GH (Chiodera et al. 1984) and of TSH (Fawley et al. 1985). A more generalized inhibitory role of OT in endocrine regulation, like that of somatostatin for example, is possible.

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References


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Note added in proof: While this text was in press, another group has shown an inhibitory role of oxytocin on corticotrope function in the human (Suh et al. 1986, Neuroendocrinology 44: 309–313).