Inhibitory effect of exogenous oxytocin on ACTH and cortisol secretion during labour

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Summary

Complex mechanisms which are still not completely defined, are responsible for the spontaneous onset of labour: an essential role is attributed to endocrine factors.

A massive increase, even three times higher than normal physiological values of ACTH and cortisol, has been reported during labour.

Similar behaviour has also been recorded for oxytocin at the end of pregnancy as well as during labour.

The relationship between oxytocin and the adrenal axis are still debated thus the goal of our study was to attempt to clarify this rapport.

Sixty-two women at the end of a term-pregnancy agreed to participate in this study: 46 were innoculated with oxytocin (syntocinon) every 20 minutes for 1 hour; 16 were administered a natural placebo every 20 minutes for 1 hour (control group).

ACTH and cortisol values from plasma samples were taken every 20 minutes and analyzed. Our results demonstrated an inhibitory effect of exogenous oxytocin on ACTH and cortisol release.

This inhibitory effect, as shown by our results, is time and dose-related. High oxytocin levels, as during exogenous infusion, could induce an effect opposite a normal physiologic one.

Key words: Oxytocin; Labour; Adrenal axis.

Introduction

Complex mechanisms, which are still not completely defined, are responsible for an increase in spontaneous labour. In addition to mechanical and nervous factors, an essential role is attributed to endocrine factors [1-3].

As neuroendocrine correlations are not clear, oxytocin and the adrenal axis have been closely observed throughout pregnancy as well as during labour [1-3].

In fact they both show relevant variations throughout pregnancy as well as during labor [1-3].

During pregnancy, plasma ACTH levels show a progressive increase without exceeding normal values, in spite of what happens to cortisol. In fact blood cortisol levels gradually rise, reaching values two times higher than the ones in healthy non-pregnant subjects during the last trimester of pregnancy [1-3].

A massive increase, even three times higher than normal physiological values of ACTH and cortisol, has instead been reported during labour [3].

Similar behaviour has also been recorded for oxytocin at the end of pregnancy as well as during labour [4].

Oxytocin is the first peptide hormone to be sequenced and synthesized; traditionally it has been considered to act on only the uterus and breast.

Oxytocin stimulates uterine contractions especially during pregnancy. High oxytocin doses could induce the start of labour or its acceleration. Oxytocin also induces milk production through myoepithelial breast cell contractions.

Revised manuscript accepted for publication February 22, 1999

Oxytocin is normally synthesized in the magnocellular neurons of hypothalamic nuclei as a precursor molecule and is transported axonally in association with its neurophysin to the neurohypophysis where it is released into the circulation [5].

It is clear from immunohistochemical studies that the oxytocin-secreting neurons are different from those which produce vasopressin and the regulatory mechanisms are also quite distinct [5].

Oxytocin also has no classical functions that have been well studied in the last ten years. It is well recognized that arginine vasopressin stimulates the release of ACTH both in vitro and in vivo, so that vasopressin is regarded as an important cofactor in the regulation of ACTH secretion [5-9]. The hypophysiotrophic action of oxytocin is, however, less well established.

It has been shown that, in vitro, oxytocin enhances the release of ACTH from dispersed rat anterior pituitary cells in a dose-dependent manner [1, 2, 5].

The relationship between oxytocin and the adrenal axis are still debated the goal of our study was to help clarify this relationship: the results are quite contradictory.

Some authors have not reported any variation of ACTH secretion in response to infusion [4], while others have suggested that oxytocin might to able to increase ACTH secretion [6, 7]. Finally other authors have hypothesized an inhibitory influence of oxytocin on adrenal secretion [10-14].

We do not yet know the mechanisms that regulate such an influence; many hypotheses have been suggested about oxytocin regulation of adrenal axis hormones but few data are available.

Table 1. — *ACTH and cortisol plasma levels in group I (46 women).*

Study group syntocinon	0 time basal	20 mins. 10 mUI/m*	40 mins. 20 UI/m*	60 mins. 30 mUI/m*
ACTH (pg/mL)	59.6±4.9	54.9±7.1	57.8±8.8	32.0±8.3
Cortisol (pg/mL)	42.6±5.8	44.6±6.4	42.5±6.0	40.7±7.2

Table 2. — *ACTH and cortisol plasma levels in group II (16 women).*

Control group natural placebo	0 time basal	20 mins.	40 mins.	60 mins.
ACTH (pg/mL)	55.8±6.6	60.7±4.6	55.8±4.8	58.7±6.3
Cortisol (mcgr/dL)	38.9±3.1	42.1±4.8	40.1±5.6	41.9±4.4

In fact, several chemical mediators have been shown to be responsible for the oxytocin effects on the pituitary parenchyma inhibiting ACTH and cortisol secretion, respectively, in a direct and indirect way [6, 7, 14].

The inhibitory actions carried out by exogenous oxytocin on the adrenal axis are both anatomically correlated [5-7, 10, 11].

Such a finding justifies one of the most interesting results of our study: "oxytocin inhibitory effect on ACTH and cortisol secretion is time and dose-related".

Our study is an account of the fundamental role developed by the adrenocorticotropic axis and oxytocin in the physiological onset of labour.

We evaluated the dose-related exogenous oxytocin infusion on the adrenocorticotropic axis in women at the end of a normal term pregnancy.

Materials and Methods

Patient study

Sixty-two women at the end of a normal term pregnancy agreed to participate in this study and gave their written consent. They were divided into two different groups:

Group I, consisting of 46 women, was innoculated with oxytocin (Syntocinon) for 1 hour.

Group II, consisting of 16 pregnant women, was administered a placebo solution for 1 hour (control group).

Oxytocin infusion

Forty-six women were innoculated with synthetic oxytocin.

Labour induction by syntocinon infusion is widely reported both experimentally and clinically.

Syntocinon is metabolized in the liver and kidneys; its half-life lasts a few minutes. Oxytocinase is a serum enzyme able to inactivate syntocinon but the concentration gradually reduces during pregnancy.

Syntocinon infusion was performed by dividing the hour of administration of the drug in three parts every 20 minutes.

1) Starting dose was 10 mUI/m'.

2) After 20 minutes the dose was doubled to 20 mUL/m'.

3) In the last 20 minutes syntocinon administration reached a dose of 30 mUI/m'.

Blood abstraction

The drawing of blood was performed with a particular procedure to avoid stress induced by drawing. Patient stress may cause elevated ACTH and cortisol levels.

Usually, normal procedures for blood drawing give rise to an unadvised increase of ACTH and cortisol that could distort data.

Blood collection

Polyethylene test tubes containing ethylene diamine tetraacetate disodium (EDTA) were kept in an ice-cold water bath.

Five ml of blood was collected from each antecubital vein by a heparinized syringe and immediately transferred to an ice bath. The blood was centrifuged at 4 °C for 15 minutes at 1,500 g (rpm), and the plasma was separated and kept at -20 °C until it was assayed.

Experimental procedure

ACTH and cortisol values from plasma samples taken every 20 minutes were determined with a specific R.I.A. kit. All samples from each protocol were measured in the same assay to avoid inter-assay variability.

The mean intra-assay coefficients of variation were 5,2% and 6.1%, respectively, for ACTH and cortisol.

ACTH assay

Plasma ACTH was measured by immunoradiometric assay provided by INCSTAR (INCSTAR Corp., Stillwater, Minnesota, U.S.A.). This kit is used for the quantitative determination of human adrenocorticotropic hormone (ACTH) in EDTA plasma.

This is accomphished using two ACTH antibodies, each one specific for different regions of ACTH molecules; the iodinated tracer used in this system contains a purified polyclonal goat antibody specific for ACTH 26-39 and an iodine 125-labeled monoclonal antibody specific for ACTH 1-17.

Only ACTH 1-39, present in the sample, bind to both antibodies to form an antibody complex. We measured the radioactivity bound to the bead.

Concentrations of ACTH present in the sample are directly proportional to the radioactivity measured.

Results have been calculated by comparing the CPMs of each sample to the CPMs for the ACTH standards.

Cortisol assay

Plasma cortisol was measured with a radioimmunoassay kit provided by ORIOIN Diagnostica (ORIO Corporation, Finland); it is a quantitative test designed for direct in vitro measurement of cortisol in human serum.

The assay involves the use of a cortisol antiserum which has a negligible cross-reactivity with other endogenous corticosteroids and cortisol metabolites. Binding of cortisol to serum proteins (mostly cortisol binding globulin) is inhibited by the inclusion of hormone analogues in the tracer solution. Final separation of free and antibody-bound hormone is performed by precipitating the latter with polyethylene glycol.

The sensitivity of this method, defined as the smallest detectable concentration and equivalent to twice the standard deviation of the zero-binding value, is approximately 4-7 nmol/1.

As to specificity, cross-reactivity of the antiserum is 100% for cortisol, 29% for prednisolone and 5.2% for 5B-dihydrocortisol; it is not prominent for other steroids.

Results were calculated drawing a standard curve on semi-log graph paper with B/B° % on the ordinate and cortisol concentrations (nmol/1) of the standards on the abscissa.

Statistical analysis

Results are expressed as the mean ±SEM (standard error of the mean).

The Student's t-test for unpaired data was used to compare values measured in the control group with those obtained from group I.

Our determinations did not present "inter-assay" variations because they were carried out in the same assay. "Intra-assay" variation media were 5.2% and 6.1%, respectively, for ACTH and cortisol.

Results

As already indicated in the preceeding paragraph, the determination of ACTH and cortisol levels were done repeatedly in the course of the experiment in the same way as the increase of the syntocinon dose was done each 20 minutes.

Basal ACTH media registered for Group I (46 patients) and the Control Group (16 patients) were in the upper normal range. However, they were in the normal physiological range. In fact ACTH media levels were 59.6±4.9 pg/ml for the first group (syntocinon administration) and 55.8±6.6 pg/ml for the control group.

Basal cortisol plasma levels were definitely higher than the normal range in both groups. In fact they were 42.6±5.8 mcrg/dl and 38.9±3.1 mcrg/dl, respectively, for group 1 and the control group.

Such results show that blood cortisol levels gradually rise throughout pregnancy reaching values two times higher than in normal pregnancy during the last trimester. ACTH also shows a progressive increase throughout pregnancy but ACTH plasma levels do not exceed normal values.

ACTH and cortisol plasma levels obtained during the experiment are reported in Tables I and II, respectively, for group I and the control group.

Discussion

Our results demonstrate an inhibitory effect of exogenous oxytocin on ACTH and cortisol release, as already reported by other authors [10-14].

Comparing the first group's data with those relative to the control group it is easy to observe that the inhibitory effect seems to be time and dose-related. In fact the ACTH serum level decreases only after 60 minutes, while cortisol serum levels also decrease, though not significantly. ACTH and cortisol inhibition by exogenous oxytocin is widely demonstrated our study.

It is clear that exogenous oxytocin reduced hormone levels in group I versus the control group.

As the inhibitory effect of exogenous oxytocin is remarkable only after 60 minutes (when the syntocinon dose is 30 mUI/min), we can confirm that it is time and dose-related.

ACTH and cortisol values of the intermediate abstractions demonstrate that in these two breaks of time the syntocinon inhibitory effect is still not evident. Such a finding should make us consider that oxytocin therapy is responsible for the complex variation of hormonal levels, some of which are time and dose-related.

The mutual relations between oxytocin and the adrenal axis are still debated. Some authors have suggested that oxytocin might be able to increase ACTH secretion [6, 7], while other authors have hypothesized an inhibitory influence of oxytocin on adrenal secretion [10-14].

This latter hypothesis, reinforced by our results, supports the existence of an inhibitory effect of exogenous oxytocin on ACTH and cortisol secretion to confirm the existence of neuroendocrine correlation mechanisms and the effectiveness of non-classical oxytocin functions [5].

Oxytocin has also been found to increase CRH-mediated ACTH secretion in vivo and passive immunoneutralization of oxytocin decreases the ACTH response to stress [5, 15].

However, it is clear that there are many correlations between the three considered neuropeptides; CRH, oxytocin (OT) and vasopressin (AVP). These correlations have been demonstrated in rats and rhesus monkeys [5, 16].

Our data demonstrate that during labour exogenous oxytocin induces a decrease in cortisol and ACTH serum levels. This inhibitory effect, as shown by our results, is time and dose-related. In fact ACTH serum levels only decrease after 60 minutes and at an average of 50% [17-19]. Cortisol serum levels also decrease, though not significantly.

Oxytocin and ACTH receptors have been anatomically (perikarya of the paraventricular nuclei) and functionally (high affinity oxytocin receptors) demonstrated. Such receptors could be responsible for the oxytocin inhibitory effect.

High oxytocin levels, such as exogenous infusion, could induce an effect opposite a normal physiologic one. In addition, inhibitory feed-back could be involved in ACTH regulation.

Other authors suggest that there is not any receptorial correlation between oxytocin and ACTH regulation and they support the implication of some neurophysins [14].

Some authors showed that the affinity of oxytocin for AVP rat anterior pituitary receptors was only 1.1% that of AVP suggesting that a competition at that level could possibly be observed for very high OT/AVP ratios [14].

Other laboratory experiences showed low oxytocin affinity for AVP receptors and gave value to neurophysin hypotheses, particularly hNpl and hNp2 [11, 14].

An action of exogenous oxytocin on endogenous AVP or oxytocin release also would seem less likely, since, in the present work there was no systematic modification of hNp1 and hNp2 in the course of oxytocin infusion [14] . The increased fluctuation of both neurophysins during the perfusion indicates, however, that some kind of irregularity in endogenous neuropituitary release could occur [14].

The blocking action of oxytocin on ACTH release could be due to oxytocin itself or to a smaller product of biodegradation such as the MSH-inhibiting factor; the terminal tripeptide of oxytocin (Pro-Leu-Glycinamide) is unknown [12].

There are many differences between these two different theories but together they demonstrate an oxytocin and ACTH correlation.

Our study contribute to the understanding of the mutual relations between oxytocin and the adrenal axis that are still debated.

Cortisol inhibition by exogenous oxytocin is not a direct effect of the neuropituitary hormone. However, it is probably an indirect aspect of ACTH inhibition [20].

Such a hypothesis is confirmed by a lower reduction of cortisol plasma levels after 60 minutes of infusion. This inhibitory effect may be lower but longer lasting than the ACTH one [20].

Oxytocin has hypophysiotrophic actions which in animals are stimulatory for prolactin, gonadotrophins and ACTH release, but in men the action of ACTH secretion is inhibitory [5].

Cortisol regulation could be paracrine to confirm differences we have found between cortisol and ACTH in our work.

These differences are new factors derived from interest in cortisol and ACTH because before our study other authors evaluated only ACTH [10, 14].

Preceeding studies were performed on male subjects [12-14] and not on pregnant women as we have done.

In conclusion, the inhibitory effect of exogenous oxytocin on the adrenocorticotropic axis in nonpregnant women reported by some authors in normal subjects is confirmed by our results in women also at term pregnancy.

Such a finding should make us consider that oxytocin therapy is responsible for the complex variation in hormonal levels, some of which are time and dose-related.

Surely the consequences of such modification on the normal physiological mechanisms of labour require further investigations, thus it will be necessary to conduct further studies to answer the questions and to obtain ultimate results.

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