Effects of rat corticotrophin-releasing factor, arginine vasopressin and oxytocin on the secretions of adrenocorticotropic hormone and corticosterone in the fetal rat in late gestation: in vivo and in vitro studies

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Deloof S, Montel V, Chatelain A. Effects of rat corticotrophin-releasing factor (rCRF), arginine vasopressin (AVP) and oxytocin (OT) were investigated in vivo in 21-day-old rat fetuses injected through the umbilical vein and in vitro on perfused anterior pituitary glands from 21-day-old rat fetuses. In vivo, rCRF (1.25 pmol·50 μl−1·fetus−1), AVP (5 pmol·50 μl−1·fetus−1) alone and rCRF in association with AVP or oxytocin (12.5 pmol·50 μl−1·fetus−1) increased plasma adrenocorticotropic hormone (ACTH) and corticosterone levels only 30 min after the start of injection. During the first 10 min of the sampling period, the injection of these peptides alone or in combination and the injection of saline decreased the plasma ACTH concentration, which was lower than that of un.injected fetuses, but had no effect on the plasma corticosterone concentration. In vitro, the release of ACTH by perfused anterior pituitary glands was increased strongly by rCRF (4 pmol/0.5 ml) but only slightly by AVP (92 pmol/0.5 ml) and oxytocin (198 pmol/0.5 ml). Arginine vasopressin and oxytocin potentiated the release of ACTH stimulated by rCRF in vitro but not in vivo. Our results suggest that rCRF is the major peptide that controls ACTH secretion in the fetal rat at term. In conclusion, the rise of the ACTH level observed only 30 min after injection of rCRF or AVP suggests the existence of a factor able to inhibit the ACTH response after injection of these peptides. This factor might be elicited by the blood volume expansion.

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It is well established that the activity of the pituitary-adrenal axis is regulated by several peptides, including rat corticotrophin-releasing factor (rCRF), arginine vasopressin (AVP) and oxytocin (OT), which are found in high concentrations in the pituitary portal blood of the adult rat (1).

In the adult rat, rCRF, which is the most potent of these peptides, increased ACTH secretion in vivo (2) and in vitro (3). A number of in vivo (4) and in vitro (5, 6) studies have shown that AVP weakly stimulates ACTH release and potentiates the ACTH response to rCRF. In addition to AVP, OT also stimulates weakly ACTH release in vivo (7) and in vitro (8) but potentiates the rCRF-stimulated ACTH release both in vivo and in vitro (9, 10).

As far as fetuses are concerned, most results are related to fetal ovine. Indeed, many in vivo studies have shown that rCRF or AVP given alone increase the plasma concentration of ACTH and glucocorticoids (11) and, given together, these two peptides present a synergistic interaction on ACTH secretion (12). In the fetal baboon, the infusion of rCRF increases plasma ACTH concentration but not cortisol (13); in vitro, rCRF is a potent stimulus of ACTH release by human fetal pituitaries (14).

In the fetal rat at term, the presence of immunoreactive rCRF, AVP or OT detected in the hypothalamus and pituitary gland (15, 16) suggests that these peptides could play an important role in the regulation of ACTH secretion by the fetal pituitary gland. Little is known of the role of these peptides on the activity of the pituitary-adrenal axis during the fetal life of the rat. Some in vitro experiments have shown that the fetal pituitary gland of the rat is responsive to exogenous CRF from day 17 of gestation and rCRF or AVP alone or in combination stimulates in vitro ACTH release from 17-, 19- and 21-day-old fetuses (17). In the present study we have examined the in vivo ACTH and corticosterone responses to rCRF, AVP or OT injected intravenously either alone or in combination on 21-day-old rat fetuses and also the in vitro ACTH response to rCRF, AVP or OT infused alone or in combination on perfused anterior pituitary lobes from 21-day-old rat fetuses.

Materials and methods

Materials

The chemical products and hormones used in this study were obtained from the following sources: Na[125]I (IMS-
30) and 1,2,6,7-[3H]corticosterone (103 Ci/mmol) from Amersham International, Amersham, Bucks, UK; oxytocin from Bioproducts, Nanterre, France; arginine vasopressin (bovine) and CRF (human, rat) from Bachem Interchim, Paris, France; ACTH antibody (no. 994) from Professor C Oliver (Faculté de Médecine, Marseille, France); ACTH(1-39) from Ciba-Geigy, Basel, Switzerland; ACTH Kit (ACTH K-PR) from Compagnie Oris Industrie-SA, Gil-sur-Yvette, France; corticosterone, L-ascorbic acid and bovine serum albumin fraction V (BSA) from Sigma, St Louis, MO, USA; aprotinin from Behring, Puteaux, France; isoctane and ethylacetate from Merck, Nogent-sur-Marne, France.

Animals and treatments

Experiments were performed on Wistar rats bred in the laboratory. Females were mated with a male for 1 night. The following day was taken as day 0 of pregnancy if spermatozoa were found in vaginal smears. Pregnant females were isolated into individual cages 24 h before being sacrificed. On day 21 of gestation the mothers were killed by a blow on the head, bled rapidly at the throat level and the fetuses were delivered rapidly by caesarean section between 08.00 and 10.00 h and kept at 37°C with their placenta and umbilical cord. The 21-day-old rat fetuses from several litters were divided into six groups receiving, respectively, rCRF (1.25 pmol·50 µl−1·fetus−1), AVP (5 pmol·50 µl−1·fetus−1), OT (12.5 pmol·50 µl−1·fetus−1), rCRF in association with AVP or OT (50 µl/fetus), all dissolved in 50 µl of saline (0.9% (w/v) NaCl) containing 0.25% BSA (w/v) through the umbilical vein. The fetuses of the last group, which received only 50 µl of saline through the umbilical vein, served as controls. Immediately after the injection, the umbilical cord of the fetuses was clamped near the umbilicus to avoid loss of blood and a possible role of placental CRH on the fetal pituitary gland. The injected fetuses were kept at 37°C and killed by bleeding at the trunk level 5, 10 or 30 min after the start of injection. Some un.injected fetuses were bled immediately after their mother’s sacrifice and served to determine basal ACTH and corticosterone values. To obtain one plasma sample it was necessary to pool the blood from three or four fetuses.

Treatment of blood samples

Blood samples were collected in polyethylene tubes containing 5% (w/v) EDTA (10 µl for 0.5 ml of blood) and aprotinin (250 units for 0.5 ml of blood). The blood samples were centrifuged at 5000 g for 10 min at 4°C and the plasma samples were stored at −80°C until assayed for ACTH and corticosterone.

Perfusion studies

Pituitary glands were removed from 21-day-old fetuses. Anterior and neurointermediate lobes were separated using stainless-steel needles under a dissecting microscope. Ten or twelve anterior lobes placed in a 1-ml chamber with a functional volume of 0.3 ml were perfused at 37°C under a constant flow rate (6 ml/h) with Krebs–Ringer bicarbonate buffer (KRBG) at pH 7.4 containing 0.2% (w/v) glucose, 0.1% (w/v) BSA, 0.018% (w/v) ascorbic acid and 0.003% (w/v) bacitracin gassed with 95% O2/5% CO2. After a 2-h equilibrium period to obtain basal production of ACTH, the anterior lobes were stimulated for 5 min with rCRF (2 pmol/0.5 ml), AVP (92 pmol/0.5 ml) or OT (198 pmol/0.5 ml) alone or CRF in combination with AVP or OT. Between each pulse, the anterior lobes were rinsed with KRBG–BSA for 60 min. Eluate fractions (0.5 ml) were collected and kept at −20°C until assay of ACTH.

Dose response of ACTH secretion to rCRF, AVP or OT

Injections into the umbilical vein of 21-day-old fetuses and perfusions of anterior lobes with increasing concentrations of rCRF, AVP or OT were performed to evaluate the concentration of these peptides required for half-maximal ACTH secretion. Adrenocorticotropic hormone was determined in plasma samples and eluate fractions.

Assay of ACTH

Adrenocorticotropic hormone was measured in unextracted plasma by radioimmunoassay (RIA) using the ACTH Kit (ACTH K-PR). The antiserum raised in rabbit by injection of ACTH1-24 coupled to BSA cross-reacted 100% with human ACTH1-39, 0.1% with β-lipotrophin hormone (β-LPH) and α-melanocyte-stimulating hormone (α-MSH) and 0% with β-MSH, γ-LPH, α- and β-endorphin, met-enkephalin and somatostatin. Assays were performed in duplicate on 0.1 ml of fetal plasma. The intra-assay variability was 4.30% (N=9) and the interassay variability was 11.73% (N=9).

Adrenocorticotropic hormone was determined in eluate fractions by an RIA performed in our laboratory. Synthetic human ACTH1-39, was labelled with Na[125I] as described previously by Hunter and Greenwood (18). The specific activity was 562 ± 13 Ci/µg (N=12). The antiserum (no. 994) obtained from rabbit following injection of ACTH1-24, conjugated with BSA was used at a final dilution of 1/500 000. The percentage of cross-reactions was 100% with human ACTH1-39 and ACTH1-24 and 0% with α-MSH, β-MSH, β-LPH, ACTH4-10, ACTH11-24, ACTH35-39 and corticotrophin-like intermediate lobe peptide (CLIP). Assays were performed in duplicate on 0.1 ml eluate fractions. The intra-assay variability was 3.22% (N=18) and the interassay variability was 9.62% (N=15).
Assay of corticosterone

Corticosterone in plasma was extracted as follows. Plasma samples (0.1 ml) were defatted with isooctane (0.5 ml) and then corticosterone was extracted with ethylacetate (2 ml). This solvent containing corticosterone was evaporated to dryness at 37°C under nitrogen and the residue was resuspended in assay buffer. The recovery of a known amount of corticosterone was over 95%.

Concentrations of corticosterone were determined by a competitive protein-binding radioassay using plasma from adrenalectomized female rats as the source of corticosteroid-binding globulin. 1,2,6,7-[3H]-corticosterone as the labelled hormone and corticosterone as the standard. The percentage of cross-reactions was 100% with corticosterone, 23% with hydrocortisone, 8% with deoxycorticosterone, 2.5% with progesterone, 0.3% with testosterone and aldosterone and 0% with pregnenolone, oestradiol-17β and dehydroepiandrosterone. Assays were performed in duplicate on 0.1-ml fractions. Intra- and interassay variabilities were 1.70% and 7.53% (N = 10 and 8), respectively.

Statistical analysis

The results are presented as means ± SEM. The unpaired Student’s t-test was used to compare the means of the different values.

Results

Effects of graded doses of rCRF, AVP or OT on ACTH concentration

In vivo and in vitro, rCRF and AVP increased the release of ACTH in a dose-dependent manner, but AVP was less potent than rCRF. The concentrations of rCRF and AVP required for half-maximal ACTH secretion were, respectively: in vivo, 1.25 and 5 pmol/50 µl; in vitro, 4 and 92 pmol/0.5 ml (Fig. 1). These values were used routinely for in vivo and in vitro experiments. Increasing concentrations of OT had no significant effect on ACTH secretion either in vivo or in vitro (data not shown). The 12.5 pmol/50 µl and 198 pmol/0.5 ml concentrations of OT, which were approximately twofold higher than AVP concentrations, were used for in vivo and in vitro experiments, respectively.

In vivo study

Plasma ACTH. During the first 10 min after injection of NaCl, rCRF, AVP or OT, or rCRF in combination with AVP or OT, plasma ACTH concentrations were lower than that of un.injected fetuses (p < 0.05–p < 0.001) (Fig. 2a) and these peptides alone or in combination did not induce any significant change in plasma ACTH concentration compared to saline injection (p > 0.05) (Fig. 2a). At the end of the sampling period the injection of saline increased the plasma ACTH concentration slightly and it was not significantly different from that of un injected fetuses (p > 0.05) (Fig. 2a). Oxytocin did not modify the plasma ACTH concentration compared to that of injected fetuses with saline (p > 0.05) (Fig. 2a) but the injection of rCRF or AVP alone or rCRF in combination with AVP or OT increased the plasma ACTH concentration compared to saline injection (p < 0.05–p < 0.001) (Fig. 2a) and un injected fetuses (p < 0.05–p < 0.001) (Fig. 2a). Arginine vasopressin or OT did not potentiate an ACTH response to rCRF (p > 0.05) (Fig. 2a).

Plasma corticosterone. During the first 10 min after injection of NaCl, peptides alone or in combination, plasma corticosterone concentrations were not significantly different from that of un injected fetuses (p > 0.05)
Fig. 2. Effect of rat corticotrophin-releasing factor (rCRF), arginine vasopressin (AVP) and oxytocin (OT) in vivo on (a) ACTH and (b) corticosterone concentrations in 21-day-old rat fetuses. Rat CRF (1.25 pmol/50 µl per fetus) (hatched bars), AVP (5 pmol/50 µl per fetus) (cross-hatched bars), OT (12.5 pmol/50 µl per fetus) (solid bars), rCRF+AVP (horizontally striped bars) and rCRF+OT (dotted bars) dissolved in NaCl (0.9% w/v) were injected intravenously through the umbilical vein. Fetuses injected with saline (50 µl/fetus) served as controls (open bars) and uninjected fetuses served to measure the basal concentrations of ACTH and corticosterone (vertically striped bars). Injected fetuses were killed 5, 10 and 30 min after the start of injection. Vertical bars represent means ± SEM, with the number of plasma samples at the base. *p<0.05, **p<0.01 and ***p<0.001 compared to uninjected value (unpaired Student’s t-test). *p<0.05; **P<0.01 and ***p<0.001 compared to saline values (unpaired Student’s t-test).
Fig. 3. Effect of arginine vasopressin (AVP) (92 pmol/0.5 ml), oxytocin (OT) (198 pmol/0.5 ml) and rat corticotrophin-releasing factor (rCRF) (4 pmol/0.5 ml) alone and in combination on in vitro ACTH release by anterior pituitary glands from 21-day-old rat fetuses perfused for 5 min. Between each pulse, the anterior pituitary glands were washed with Krebs-Ringer bicarbonate buffer for 60 min. Each profile represents the mean ± SEM of six (a) and five (b) independent perfusion experiments. The insets show the release of ACTH expressed in pmol/pituitary gland.

(Fig. 2b) and these peptides alone or in combination did not induce any significant change in plasma corticosterone concentration compared to injected fetuses with saline (p > 0.05) (Fig. 2b).

At the end of the sampling period the injection of saline increased the plasma corticosterone concentration slightly and it was not significantly different from that of uninjected fetuses (p > 0.05) (Fig. 2b). Oxytocin did not modify the plasma corticosterone concentration compared to that of injected fetuses with saline (p > 0.05) (Fig. 2b). The injection of rCRF or AVP alone or rCRF in combination with AVP or OT increased the plasma corticosterone concentration compared to saline injection (p < 0.05–p < 0.001) (Fig. 2b) and uninjected fetuses (p < 0.01–p < 0.001) (Fig. 2b). Arginine vasopressin or OT did not potentiate a corticosterone response to rCRF (p > 0.05) (Fig. 2b).

In vitro study

The production of ACTH by perifused fetal anterior lobes was stimulated by rCRF (Fig. 3). Arginine vasopressin
and OT had a weak stimulating effect on ACTH release compared to rCRF (Fig. 3). The average ACTH release (area under the curve) showed that AVP and OT potentiated the effect of rCRF on ACTH release (insets in Fig. 3).

Discussion

The present study shows that a bolus injection of saline into the umbilical vein of 21-day-old rat fetuses decreases ACTH secretion during the first 10 min. This result contrasts with data reported by most investigators, who have shown that ACTH secretion was not modified significantly after intravenous saline injection in many species such as sheep (19), rats (20), monkeys (21), men (22), fetal and neonatal sheep (11, 12). The decrease of ACTH secretion could be due to a transfer of maternal stress or by the dissecting procedure itself. However, the stability of plasma corticosterone concentrations measured in our fetuses after saline injection is not significantly different from that of un.injected control fetuses. This result seems to rule out stress coming from the mothers, because they are rapidly killed and bled, or from the handling of the fetuses during the caesarean section. Under these conditions another hypothesis can be considered to explain the decrease of ACTH secretion during the first 10 min. Indeed, a volume of 50 µl injected into the umbilical vein of 21-day-old fetuses induces a blood expansion of approximately 10–15%. Many reports have shown that blood volume expansion stimulates atrial natriuretic factor (ANF) release from atrial cardiocytes and increases rapidly the ANF concentration in the blood of adult rats (23), human fetuses (24) and ovine fetuses (25). In the rat, ANF was found to inhibit ACTH secretion in vivo (26) and to inhibit in vitro cultured anterior pituitary cells stimulated by CRF (27) but had no effect on in vitro corticosterone secretion from isolated adrenal cells (28, 29). Moreover, the apparent short half-life of ANF in rat blood (30) can explain the increase of basal ACTH level observed as early as 30 min after saline injection. Besides, the failure of the stimulating effect of rCRF or AVP alone or in combination on ACTH concentration during the first 10 min after injection also can be explained by the release of ANF by the blood volume expansion because it was shown in the rat that ANF inhibits ACTH secretion stimulated by rCRF (27). In the fetal rat, immunological studies have revealed the presence of immunoreactive ANF in both atrial and ventricular cardiocytes (31) and in the blood circulation (32). Unfortunately, ANF concentrations were not measured in the plasma of our injected fetuses and we cannot draw conclusions about the involvement of ANF in our present study. If this interpretation in relation to ANF is speculative, there is certainly good evidence that ANF serves as a corticotrophin-inhibiting factor.

In the fetal rat, the rise of ACTH level observed in vivo 30 min after injection and in vitro from perfused pituitary glands after treatment with rCRF or AVP alone or in combination is in accordance with most previous data and our results clearly show that rCRF is more effective than AVP or OT. Nevertheless, in sheep, AVP is a more potent stimulus to ACTH secretion than CRF (19). According to Shen et al. (33), this difference between rat and sheep can be correlated to the density of AVP receptors, which is higher in the sheep anterior pituitary than in the rat. In pituitary corticotroph cells it was demonstrated that CRF stimulates ACTH secretion by increasing the cyclic adenosine-3,5-monophosphate pathway (cAMP), whereas both AVP and OT stimulate ACTH secretion by activating the phosphatidylinositol pathway, and that the potentiating effect of these peptides on rCRF-induced ACTH secretion could be elicited when these two pathways were co-stimulated (34). In our in vivo experiments, OT alone has no significant effect on basal ACTH concentration and does not potentiate an ACTH response to rCRF. According to the authors, as there is little (15) or no detectable immunoreactive OT (35) in the hypothalamus and pituitary gland in rat fetuses at term, it is hardly likely that OT should influence ACTH secretion in these fetuses. However, in vitro, the high concentration of OT used in our experiments potentiates rCRF-stimulated ACTH secretion. This potentiation might result from a stimulation of AVP-binding sites by OT. Indeed, this hypothesis is supported by two observations: first, specific high-affinity AVP receptors identified in the anterior lobe of the adult rat exhibit a low affinity for OT (36); second, in the anterior lobe of the adult rat high concentrations of OT (10 µmol/l) displace [3H]-vasopressin (5 nmol/l) from its binding sites (37). Arginine vasopressin potentiates an ACTH response to rCRF in vitro but not in vivo. In vivo, the slightly elevated basal ACTH and corticosterone concentrations, which could result from a situation of stress of fetuses deprived of their mothers for 30 min, could inhibit the synergistic interactions between rCRF and AVP, but this is not the case in vitro.

In conclusion, our results suggest that rCRF is the major peptide that controls ACTH secretion in the fetal rat at term but in vivo a factor elicited by the blood volume expansion could inhibit the rise of ACTH level induced by rCRF or AVP.

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References


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