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Adrenocorticotropin Secretion by Fetal Sheep Anterior and Intermediate Lobe Pituitary Cells *in Vitro*: Effects of Gestation and Adrenalectomy*

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ABSTRACT

Immunoreactive (ir) ACTH is present in the fetal sheep intermediate lobe (IL) as well as the anterior pituitary (AP). It is not clear whether fetal IL cells can secrete irACTH and if gestational age and glucocorticoids influence the secretion of ACTH from these tissues in a similar fashion. Therefore, we examined the control of irACTH secretion by IL cells, whether the responsiveness of AP and IL cells to arginine vasopressin (AVP) and CRH changes during gestation, and whether withdrawal of adrenal steroids by adrenalectomy influences AP and IL responses. Cultured pituitary cells were studied from intact fetuses at an immature (n = 5; 108 ± 5 days) and a mature (n = 8; 139 ± 0 days) stage, from mature fetuses 3 weeks after bilateral adrenalectomy (n = 6), and from neonatal lambs within 16 h of birth (n = 6). Secretion of irACTH was determined by RIA of incubation medium obtained during 3-h exposure of cells to vehicle, AVP, CRH, or both. In all cases, IL cells secreted measurable irACTH. The IL cells of immature fetuses responded to CRH (133 ± 8% increase over basal secretion), AVP (52 ± 6%), and CRH plus AVP (244 ± 8%). In contrast, IL cells from mature fetuses responded only to CRH (160 ± 20%) or CRH plus AVP (259 ± 44%), as did cells from mature adrenalectomized fetuses (CRH, 356 ± 70%; CRH plus AVP, 627 ± 100%). Secretion from neonatal IL cells was not significantly increased above basal rates by CRH and/or AVP. The AP cells from immature fetuses responded significantly to CRH (406 ± 16%), AVP (114 ± 8%), and CRH plus AVP (559 ± 38%), whereas cells from mature fetuses responded only to AVP (249 ± 40%) or to CRH plus AVP (570 ± 146%). In AP cells from mature adrenalectomized fetuses, the response pattern resembled that of immature intact fetal sheep (CRH, 429 ± 76%; AVP, 146 ± 15%; CRH plus AVP, 541 ± 94%). Neonatal AP cells responded to CRH (196 ± 25%), AVP (442 ± 71%), and CRH plus AVP (646 ± 93%). Further characterization of IL cells (n = 6 fetal and 2 neonatal) indicated that they were inhibited by dopamine (basal ACTH secretion decreased by 25 ± 4%; ACTH secretory response to CRH decreased by 32 ± 10%). These results show that fetal neurointermediate lobe cells secrete irACTH under basal and stimulated conditions. Moreover, the pattern of response of AP and neurointermediate lobe cells to secretagogues is influenced by gestational age and, possibly, cortisol. (*Endocrinology* **137:** 3394–3400, 1996)

TIMELY ACTIVATION of fetal adrenal steroidogenesis plays a pivotal role in development (1, 2). Concurrent with development of the adrenal cortex itself is the development of the regulatory system for adrenal steroidogenesis, including that of the hypothalamus and pituitary (3). Corticotrophs of the anterior pituitary (AP) are thought to be the primary, if not sole, source of ACTH in sheep and other adult mammals (4).

The pituitary undergoes marked morphological changes during development and postnatal life (5). In fetal sheep AP, two distinct types of corticotrophs have been described, one large and columnar (called fetal) and one smaller and stellate (called adult) (6). The former are preponderant at 90 days gestation (term is ~147 days), whereas the latter predominate by 130 days and into postnatal life. Infusion of cortisol in fetal sheep has been shown to cause premature predominance of adult-type corticotrophs. The relationship, if any, between the morphological development and functional changes is not known. In addition, the sensitivity of the fetal pituitary *in vivo* to hypothalamic stimulation changes as a function of gestational age (7, 8). For example, fetuses at gestational age 104–115 days are reportedly more or less sensitive to CRH than fetuses at 138–142 days (7, 8).

In sheep, as in humans, the intermediate lobe (IL) of the pituitary is most prominent in fetal life (5). The cells of the IL undergo relatively little proliferation postnatally, thereby receding into a residual presence (5).

ACTH is known as the primary regulatory factor driving synthesis of glucocorticoids (4). Synthesized as part of a much larger precursor, POMC, ACTH is formed and stored in AP cells (9). In IL cells of species with prominent ILs, ACTH is further cleaved to two peptides, αMSH and corticotropin-like IL peptide (CLIP) (9). Immunoreactive (ir) ACTH (as opposed to the smaller peptides) has been detected in fetal sheep IL cells, rendering the IL a potential second source of ACTH in the fetal sheep (10, 11).

Although both IL and AP cells synthesize POMC, they are

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regulated by different extrapituitary factors. AP cells are stimulated to the greatest extent by CRH or AVP and to a lesser extent by catecholamines, angiotensin II, and other factors (12, 13). IL cells, on the other hand, may secrete ACTH in response to CRH (14), but are thought to be primarily under inhibitory control by dopamine, as demonstrated by decreasing basal and CRH-stimulated ACTH secretion in canine intermediate lobes (15). Other factors that have no effect on AP corticotrophs may alter IL secretory function. Examples of these factors include TRH, which stimulates αMSH secretion by *Xenopus* melanotrophs (16), and somatostatin, which inhibits the secretion of POMC-derived peptides from rat melanotrophs (14). To date, few if any data on sheep IL cells have been published.

The purpose of the present study was to define the ontogeny of the pattern of ACTH responses of fetal AP and IL cells to CRH and AVP, to determine whether the fetal adrenal contributes to any developmental changes observed, and to determine whether control of ACTH secretion by IL cells more closely resembles that of AP corticotrophs or the control of αMSH secretion by melanotrophs.

Materials and Methods

Pregnant ewes with known mating dates were anesthetized with ketamine and pentobarbital. Cells obtained from fetuses of either sex were used in these studies. The fetuses were delivered at 100–115 days (n = 8; immature), 129–136 days (n = 2; intermediate), or 138–145 days (n = 16; mature) through a midline abdominal incision and killed with an overdose of pentobarbital. In six additional ewes, fetal bilateral adrenalectomy under aseptic conditions was performed at 120 days gestation, and the fetuses were delivered, as described above, at 138–146 days. Term in these sheep is about 145 days. Eight additional untouched lambs were allowed to deliver vaginally at term (neonates). The lambs were removed within 16 h of birth, anesthetized, and killed by an overdose of pentobarbital. Because the number of cells obtained from each pituitary lobe limited the number of treatments possible within the experiments, not all treatments were performed on the cells from all animals. All procedures involving animals were approved by the institutional animal care and use committee.

Tissue harvest

The fetal and neonatal pituitary glands were removed. The AP and neurointermediate lobe (NIL) tissues were gently separated from one another by blunt dissection and put into tubes containing cold HEPES dissociation buffer (HDB) (17). The IL (pars intermedia) separates from the AP along with the pars nervosa. Microscopic examination of the separated tissue confirmed complete dissection of the anterior from the neurointermediate lobes. Immunocytochemistry of similarly dissected pituitaries was also fully consistent with the above description. All cells positively staining for αMSH were in the NIL section. As the pars nervosa does not secrete ACTH, no attempt was made to separate the pars intermedia from the pars nervosa.

The AP and NIL were processed separately according to identical procedures. Tissues were minced into fragments (~1 mm^3). The fragments were washed with HDB solution and then placed in collagenase II (0.04%; Worthington Biochemical Corp., Freehold, NJ) and deoxyribonuclease I (Sigma Chemical Co., St. Louis, MO) solution in HDB. Polypropylene centrifuge tubes containing the fragments and digestion solution were gently rocked 2.5 h at 37 C. The enzymatic reaction was stopped by the addition of complete medium (CM) to a final volume of 15 ml. In these studies, all cell culture reagents were purchased from Life Technologies (Grand Island, NY). CM consisted of DMEM plus Ham's F-12 medium (1:1) to which charcoal-stripped FCS and charcoal-stripped horse serum were each added to a final concentration of 10%. The cell suspension was washed three times in the CM. The cells were plated in Costar 48-well tissue culture plates (Costar, Cambridge, MA) in a 500-μl volume, such that each well received one eighth of the total AP or NIL cell suspension. The cells were incubated in 5% CO_2 at 37 C for 3–5 days. At the end of the period, the cells were washed three times with incubation medium (IM). This consists of DMEM and Ham's F-12 medium (1:1) with 0.1% polypep (Sigma). Then the cells were incubated for 1 h at 37 C to equilibrate to serum-free conditions, after which they were again washed with IM and prepared for experimental incubation. Concentration-response curves for ACTH secretory responses to AVP and CRH (both from Peninsula, Belmont, CA) were generated in preliminary experiments. Subsequent experiments were performed in two series. In one series, duplicate wells received vehicle, CRH (10 nM), AVP (100 nM), or CRH plus AVP and then were incubated (37 C; 5% CO_2) for 3 h. In the other series, cells were incubated with vehicle, CRH (10 nM), dopamine (5 μM; Sigma), TRH (100 nM; Sigma), or CRH plus dopamine. In both series, after removal of the incubation medium, cells were solubilized in Nonidet P-40 (Sigma) detergent (0.1%) in IM for assay of cellular DNA or ACTH. The media and samples of cellular content were stored at −20 C until the time of the assays.

RIA

ACTH immunoreactivity was measured by RIA. Synthetic human ACTH-(1–39) was used in preparing standards and the iodinated tracer. The antibody used was produced in our laboratory, and on a molar basis, it reacts equally well with ovine ACTH-(1–39), human ACTH-(1–39), and ACTH-(6–24) (18). It is more than 90% reactive with ACTH-(1–24) and less than 1% reactive with ACTH-(1–17) or human ACTH-(18–39) (CLIP). The antibody recognizes the high mol wt forms of ACTH found in fetal sheep anterior pituitary extracts, although the exact cross-reactivity is not known (19). The antibody does not recognize ACTH-(1–10), ACTH-(1–10)NH_2, ACTH-(4–11), ACTH-(11–19), ACTH-(11–24), or ACTH-(25–39) fragments of the ACTH-(1–39) peptide. The sensitivity of the assay is 4.5 pg/tube, and the intra- and interassay coefficients of variation are 13% and 18%, respectively.

DNA measurements

DNA was measured by the TKO 100 Mini-Fluorometric method (Hoefer Scientific Instruments, San Francisco, CA). At the time of measurements, different dilutions of the DNA standard (calf thymus DNA standard) were made by mixing known quantities of the DNA standard with 1 × TNE (Tris base, Na_2EDTA, and NaCl) and freshly prepared capillary assay solution (10 × TNE, Hoechst 33258 stock solution in water). Experimental samples were prepared in the same way as the DNA standard. The amount of DNA in the experimental samples was calculated by interpolation of the standard curve.

Data analysis

Secretion results are reported as the mean ± SEM corrected for variability between cultures and experiments as follows: per pg DNA (when measuring responses in groups of cells obtained at the same gestational age), per ng ACTH content (when comparing responses among groups of pooled cells of different gestational ages, because of the changing proportion of corticotrophs), and as a percentage of control secretion (where responses to treatments are compared among groups of cells from fetuses of different gestational ages). The concentration-response curves (Fig. 1) were analyzed by one-way ANOVA to determine whether there was a relationship between the concentration of secretagogue and the secretion of ACTH. In the first series of experiments (summarized in Figs. 2-4), the effects of gestational age and adrenalectomy on ACTH responses to AVP and CRH treatment of AP and NIL cells were analyzed among the experiments in which all treatments (vehicle control, CRH, AVP, and CRH plus AVP) were tested in both lobes (n = 5 immature; n = 8 mature; n = 6 adrenalectomized) by two-way ANOVA. Where ANOVA indicated a significant effect of age or treatment, significant differences between individual groups were ascertained by Scheffe's test. In the second series of experiments (Fig. 6), the effects of the treatments were analyzed by one-way ANOVA; the effects of dopamine on specific responses were determined by *t* test. Significance of effect was taken at $P < 0.05$.

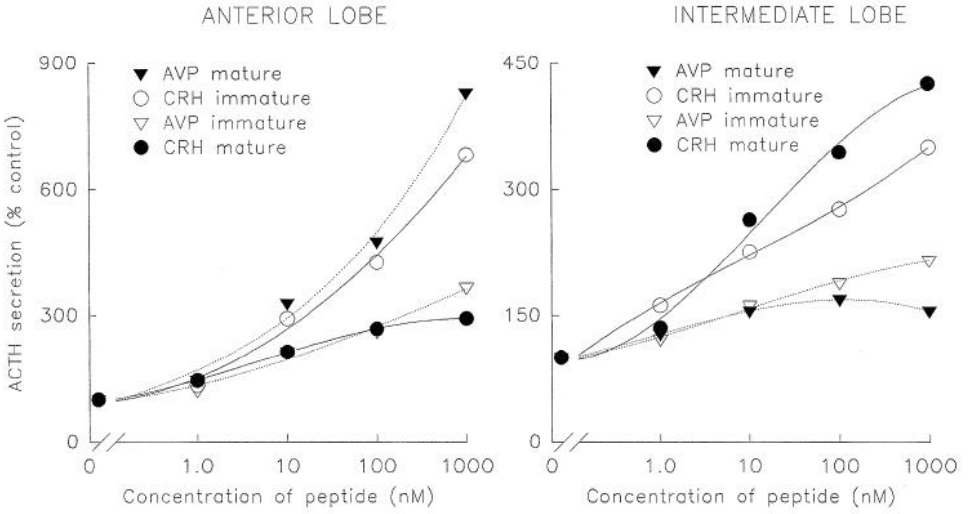

FIG. 1. Response of ovine AP (*left*) and NIL (*right*) cells obtained from immature (*open symbols*) and mature (*filled symbols*) fetal pituitaries to different doses of CRH (*circles*; 10^{-9}-10^{-6} M) and AVP (*triangles*; 10^{-9}-10^{-6} M). n = 3 experiments at each age.

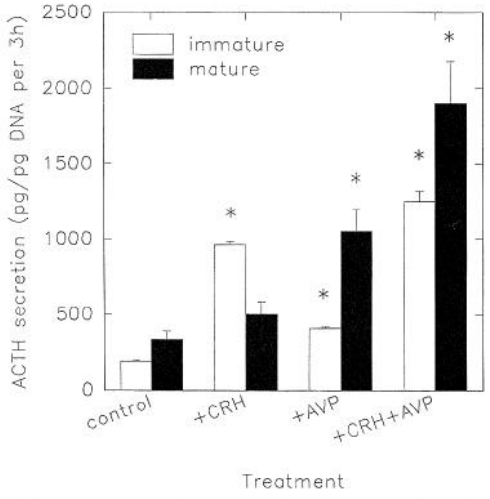

FIG. 2. irACTH secretion by cultured ovine anterior lobe pituitary cells obtained from immature (*open bars*; n = 5) and mature (*solid bars*; n = 8) fetuses in response to vehicle, CRH (10 nM), AVP (100 nM), and CRH plus AVP. *, $P < 0.05$ *vs.* respective (vehicle) control.

Results

Concentration-response relationship

The secretion of ACTH by AP and IL cells of immature and mature fetuses as a function of the concentrations of CRH and AVP is summarized in Fig. 1.

AP. Secretory responses to CRH and AVP increased with concentration in AP cells in both immature and mature fetuses. As can be seen, in cells obtained from immature fetuses, the average ACTH secretory response to CRH at 1 μM (683% of control secretion) was greater than the response to AVP (366%) at the same concentration. In contrast, in mature cells, the response to 1 μM AVP (829%) was greater than that to 1 μM CRH (293%).

NIL. IL cells behaved somewhat differently. CRH produced a concentration-dependent increase in ACTH secretion in cells from both immature and mature fetuses. There was no clear relationship between the concentration of AVP used and the ACTH response in immature and mature IL cells (note different scales on *ordinates of left and right panels*).

Responses of AP cells from intact fetuses

ACTH secretion by AP cells obtained from intact fetuses at immature and mature stages is summarized in Fig. 2.

Basal ACTH secretion. There was no statistically significant difference in ACTH secretion under basal conditions between immature (191 ± 7 pg ACTH/pg DNA·3 h) and mature (334 ± 54) AP cells.

Stimulated secretion. The response to CRH (10 nM) stimulation was different in the cells from the two age groups. Cells from immature fetuses responded to CRH stimulation with an increase in ACTH secretion (net increase over control, 771 ± 20 pg ACTH/pg DNA·3 h). In contrast, mature cells did not respond significantly to CRH (167 ± 50).

Cells from both immature (216 ± 10) and mature (717 ± 106) fetuses showed a significant response to stimulation by AVP (100 nM), with greater responses occurring in late gestation cells.

The net responses to AVP plus CRH in the cells of both groups (immature, 1062 ± 65; mature, 1569 ± 263) were significant. Statistical analysis indicated that the respective net responses to both secretagogues in combination were indistinguishable from the mathematical sums of the net responses to the individual secretagogues.

Responses of NIL cells from intact fetuses

ACTH secretion by IL cells obtained from intact fetuses at immature and mature stages is summarized in Fig. 3.

Basal secretion. Basal ACTH release from cells of mature fetuses (188 ± 27 pg ACTH/pg DNA·3 h) was statistically indistinguishable from that from cells of immature (89 ± 3) ovine fetuses.

Stimulated secretion. IL cells obtained from immature and mature fetuses responded significantly to stimulation by CRH, with the response to CRH being greater in cells from

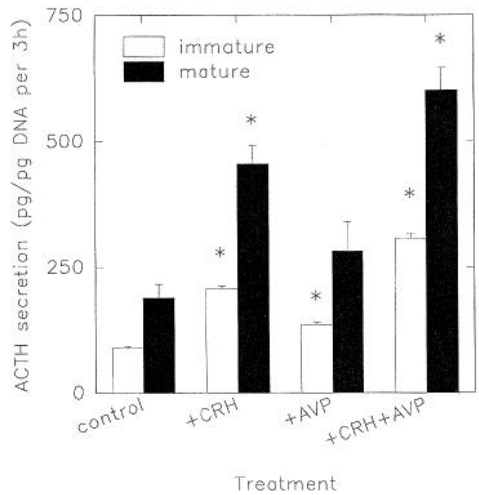

FIG. 3. irACTH secretion by cultured ovine IL cells obtained from immature (*open bars*; n = 5) and mature (*solid bars*; n = 8) fetuses in response to vehicle, CRH (10 nM), AVP (100 nM), and CRH plus AVP. *, $P < 0.05$ *vs.* respective (vehicle) control.

late (net increase, 267 ± 26 pg ACTH/pg DNA·3 h) compared to immature (116 ± 6) animals (Fig. 3).

In cells from immature sheep fetuses, there was a modest, but significant, response to AVP stimulation (net increase, 46 ± 4); ACTH secretion in the presence of AVP in cells from mature fetuses (92 ± 40) was not significantly different from basal secretion (Fig. 3).

There was a significant response to stimulation by AVP plus CRH in cells from both immature (net increase, 216 ± 7) and mature (403 ± 41) fetuses, and these respective responses were statistically indistinguishable from the mathematical sums of the net responses to CRH and AVP separately.

Responses of cells from adrenalectomized fetuses

ACTH secretion by AP and IL cells obtained from adrenalectomized fetuses is summarized in Fig. 4.

AP. The responses of AP cells from late gestation adrenalectomized fetuses resembled those of cells from intact immature fetuses. Basal secretion was 234 ± 43 pg ACTH/pg DNA·3 h. There was significant stimulation by CRH (net increase, 916 ± 76 pg ACTH/pg DNA·3 h), AVP (328 ± 49), and CRH plus AVP (1111 ± 106).

NIL. The ACTH secretory responses of IL cells from late gestation adrenalectomized fetuses resembled the responses of cells from the two intact groups, with the response to AVP (no significant increase) being less than that to CRH. Basal secretion was 146 ± 24 pg ACTH/pg DNA·3 h. Significant responses occurred in the presence of CRH (523 ± 111) and CRH plus AVP (865 ± 132).

Comparisons of responses among groups

The changing response patterns of AP and IL cells with fetal development and in response to adrenalectomy are summarized in Fig. 5.

AP cells from immature fetuses responded primarily to CRH, rather than AVP, at a higher concentration. Nearer term (mature), the relative roles of CRH and AVP became reversed, and by the time of birth, AP cells assumed the pattern we and others (13, 20) have reported for adult sheep AP cells. Withdrawal of steroids by adrenalectomy on day 120 caused the response pattern of mature gestation AP cells to resemble that of immature AP cells.

For both immature and mature pituitaries, the response patterns of IL cells were quite similar, essentially showing only a response to CRH. By the time of birth, the response capacity of IL cells appeared to be negligible, although measurable amounts of ACTH were still being secreted. Adrenalectomy on day 120 caused no qualitative change in the response pattern to CRH or AVP, but increased the magnitude of response to CRH.

Characterization of IL cells by responses to dopamine and TRH

The ACTH secretory responses of IL cells to dopamine, TRH, CRH, and dopamine plus CRH are summarized in

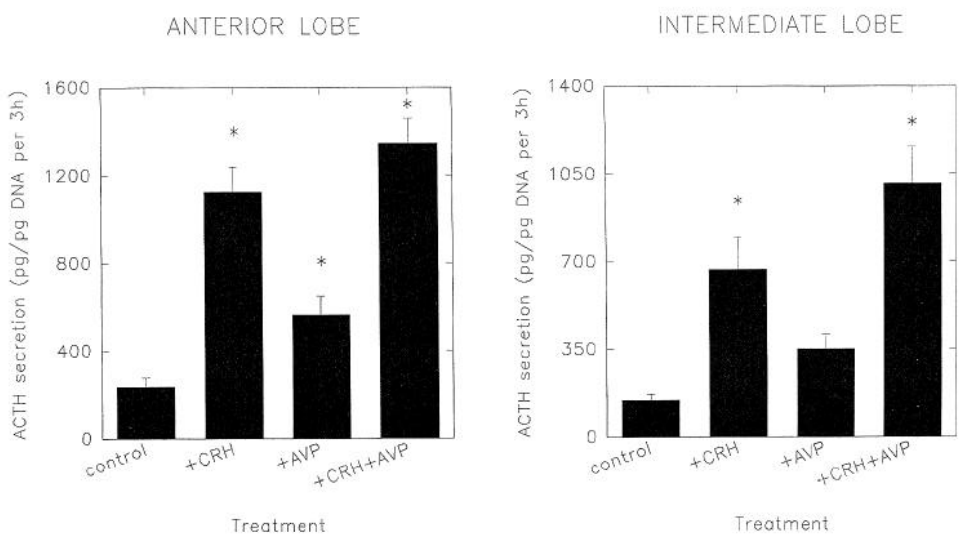

FIG. 4. Responses of cultured AP (*left*) cells and NIL (*right*) cells obtained at late gestation from fetuses adrenalectomized 3 weeks earlier to vehicle, CRH (10 nM), AVP (100 nM), and CRH plus AVP. n = 6 for both groups. *, $P < 0.05$ *vs.* (vehicle) control.

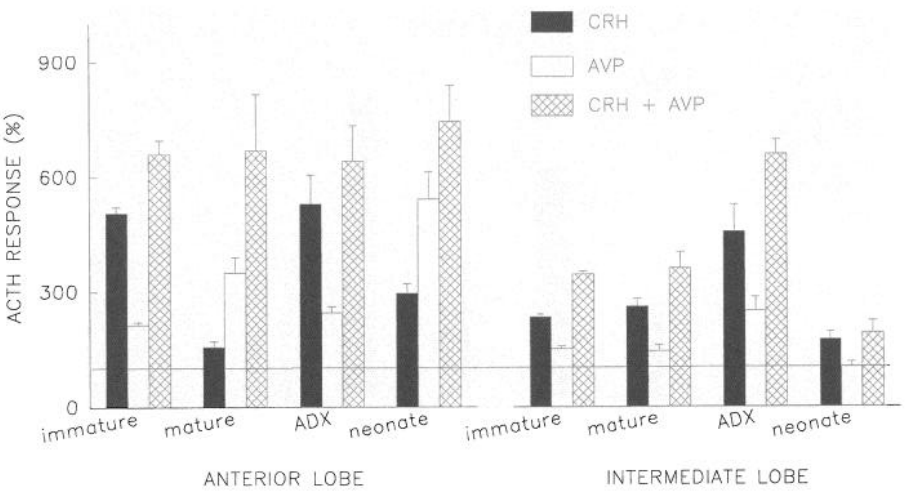

FIG. 5. ACTH secretory responses to CRH (10 nM), AVP (100 nM), and CRH plus AVP of fetal and neonatal AP (*left*) and NIL (*right*) cells, expressed as a percentage of secretion in the absence of secretagogue. n = 5, 8, 6, and 6 for immature, mature, adrenalectomized (ADX), and neonatal responses, respectively. The *horizontal line* at 100% represents no change in secretion.

Fig. 6. The apparent inhibitory effects of dopamine did not change with gestational age, and therefore, we pooled results of IL cells from several gestational ages. Dopamine consistently decreased both basal ACTH secretion and the secretory response to CRH. In IL cells, dopamine decreased basal ACTH secretion by 25 ± 4% and the ACTH secretory response to CRH by 32 ± 10%. In contrast, in AP cells, dopamine had no effect on basal or CRH-stimulated ACTH secretion. TRH had no significant effect on ACTH secretion by IL or AP cells.

Discussion

To our knowledge, these are the first data that show the ability of cultured ovine fetal IL cells to secrete irACTH and that this occurs in both immature and mature stages. The results of the present study demonstrate that fetal ovine IL cells in culture secrete ACTH (which is not due to αMSH or CLIP reactivity in the assay) under unstimulated conditions and in response to CRH. In addition, these are the first data to demonstrate that the developmental changes in the pattern of ACTH responses to CRH and AVP in the AP cells are influenced markedly by the presence of the fetal adrenal.

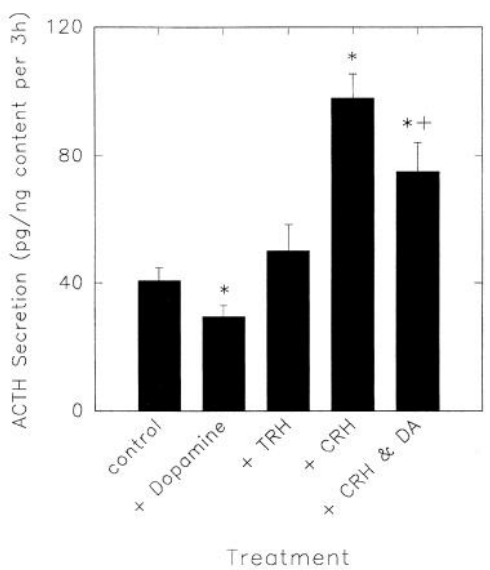

FIG. 6. ACTH secretory responses of IL cells to dopamine (5 μM), TRH (100 nM), CRH (10 nM), and CRH plus dopamine. n = 8 (pooled; 1 immature, 2 intermediate, 3 mature, and 2 neonatal). *, $P < 0.05$ *vs.* vehicle control; +, $P < 0.05$ *vs.* CRH alone.

IL cells from both immature and mature fetuses responded to CRH. Melanotrophs of a number of species have been shown to express receptors for CRH and to respond to CRH by secreting ACTH and/or MSH (14, 15, 21–24). Interestingly, AVP, at least in cells from immature fetuses, also appeared to produce a very modest ACTH secretory response in IL cells by itself and in combination with CRH. Although this may be due to some contamination of the NIL cultures with AP cells, there has been at least one other report of IL cells (rats) responding to AVP (25). In any event, the response to AVP is smaller than the response of these cells to CRH and was not increased at higher concentrations of AVP.

There was a difference in the ACTH secretory response patterns of AP and IL cells, in both the pattern of responses to CRH and AVP and the inhibitory effects of dopamine. This was not unexpected, given the different ontogenies and characterizations to date of AP and IL POMC-synthesizing cells (14, 15). The difference in secretory pattern also provides evidence that the secretion in NIL cultures was not due to gross contamination with AP cells and *vice versa*. The effects of CRH and/or dopamine on IL cells suggest that the irACTH secreted by these cultures can be characterized as a response of melanotroph, rather than corticotroph, cells.

The response of AP cells to CRH or AVP changed, dependent upon the gestational age of the fetuses. CRH (at 10 nM) produced the greater response in AP cells from immature fetuses, whereas AVP (at 100 nM) produced the greater response in mature fetal AP cells. This latter finding is consistent with a study by Brooks and Gibson (26), who found that AVP is more potent than CRH in cultured late gestation AP cells. In contrast, the laboratories of Durand *et al.* (27, 28) and Lu *et al.* (29) suggested that the ACTH response to CRH at term is greater than that to AVP. The reason for the reported differences is not readily apparent, but may relate to the tissue used, dose of secretagogue, duration of incubation, and expression of results. For example, in one laboratory (27, 28), the whole pituitary gland was used, whereas in our

study, the lobes were separated; in the other study (29), secretion was measured after a 48-h incubation period, whereas we used a 3-h incubation.

In the present study we focused on two gestational ages, one associated with relatively low and one with relatively high plasma concentrations of adrenal steroids (3) and ACTH-(1–39) (30, 31), and examined the changes in the separate lobes of the pituitary. A decrease in pituitary responsiveness to CRH *in vivo* between these two stages of gestation has also been described (7), but this finding has been called into question (8). Indeed, McFarlane and colleagues (8) suggest that, if anything, the fetal pituitary becomes more responsive to CRH between 104–108 and 138–142 days gestation. At the very least, it is reasonable to assume from these studies that over the course of gestation, the fetal sheep pituitary is subject to changes in sensitivity to hypothalamic stimulation. The transition from a CRH-predominant response to an AVP-predominant response in the present *in vitro* experiments could reflect a change in the cellular composition of the AP, with an increasing presence of corticotrophs that respond to AVP, but not CRH. AP cells of adrenalectomized fetuses showed the same pattern of ACTH response as immature AP cells, but a pattern different from that of mature AP cells. Antolovich *et al.* (6) found that adrenalectomy changes the distribution of the corticotroph population of adrenalectomized fetuses at 135 days to be similar in distribution to that of 115 day gestation fetuses (predominantly fetal type corticotrophs), but different from that of intact term fetuses (predominantly adult type). This is consistent with morphological changes being reflected in functional characteristics of the corticotrophs.

The present data indicate that transition from CRH-responsive to AVP-responsive corticotrophs may be influenced by glucocorticoids. It is tempting to speculate that glucocorticoids might hasten the transition of CRH-responsive corticotrophs into AVP-responsive cells. Alternatively, glucocorticoids might preferentially retard the proliferation of CRH-responsive cells or suppress their responsiveness. In this regard, it is noteworthy that the responses of corticotrophs to CRH are reported to be more sensitive to inhibition by glucocorticoids than are the responses to AVP (32).

The possible presence of functionally distinct corticotrophs responding differentially to secretagogues is not new (33, 34). Adult anterior pituitaries reportedly contain several types of corticotrophs, responding differentially to AVP and CRH. Thus, the present data are consistent with the possibility that in early stages of development, most corticotrophs are CRH responsive, and with advancing gestation, another class of corticotrophs appears. Recent studies from this laboratory, in which ACTH secretion from individual cells was detected by an immunoblotting method, are fully consistent with this hypothesis. The fraction of corticotrophs that can be recruited to secrete in response to CRH decreases, and the fraction that can be recruited to secrete with AVP remains high in cells from fetal sheep pituitaries harvested at increasing gestational age (35). An alternative hypothesis is that individual cells may change from CRH responsive to AVP responsive, and this process continues into adulthood. The presence of cells that respond to either CRH or AVP may represent multipotent cells or cells at a midstage between development of the previous two types of cells.

Adrenalectomy had no qualitative effect on IL cell responses. This may reflect the presence of a single type of ACTH-secreting cell in the fetal IL. As withdrawal of adrenal steroids stimulated the capacity of IL cells to respond to CRH, and birth, which follows a prolonged period of exposure to high concentrations of glucocorticoids, is associated with a diminished response of IL cells to CRH, it is possible to view glucocorticoids as exerting a direct, inhibitory, long lasting influence on IL cells. Again, one is tempted to speculate on the relationship between the fetal adrenal and intermediate pituitary glands. In one possible scenario, the pars intermedia becomes functionally active at a time when ACTH secreted by it might contribute to trophic activity at the developing adrenal. Saphier and co-workers (30), after measuring changes in plasma concentrations of various POMC-derived peptides at a number of points in gestation of fetal sheep, speculated that one role of the pars intermedia is to provide a certain measure of drive to growth and maturation of the fetal adrenal without the steroidogenesis [inherent with the trophic activity of ACTH-(1–39)] (30). Ultimately, the glucocorticoids produced by adrenals could play a role in diminishing the function of the pars intermedia. At this stage, the AP might also become more fully functional.

The concentration of CRH used in these studies for comparing responsiveness of AP cells at different ages was selected based on experiments with adult sheep AP cells (13) (our unpublished observations) and *in vivo* measurements of adult sheep portal blood CRH concentrations (36). We wanted a concentration that should produce a measurable ACTH response and be within a range that could be considered physiological. Because fetal sheep AP cells are apparently somewhat less concentration sensitive to CRH than adult cells, this concentration of CRH did not produce a maximum response, as it does in adult AP cells (26). Nevertheless, the response was measurable in fetal cells at certain gestational ages and can, therefore, be used to study changes in the responsiveness of AP cells. In any event, the purpose of the present study was to compare responses to the same treatment (*e.g.* CRH at 10 nM) among cells from the various preparations, rather than responses to treatments within a single group (comparison to vehicle control was the only exception). Only where the ACTH response to CRH (at 10 nM) was significantly greater than the response to AVP (at 10 times the concentration) were the cells explicitly described as more responsive to CRH. The concentrations of dopamine and TRH were selected as being at least equivalent to concentrations providing significant responses in other *in vitro* systems (15, 16).

In summary, this study demonstrates that fetal ovine IL cells release irACTH and respond in a pattern similar to that of adult melanotrophs of other species. Therefore, this lobe may play a role in the activation and maturation of the hypothalamo-pituitary-adrenal axis in the ovine fetus. This study also suggests that adrenal steroids may, in turn, play a role in the functional maturation of AP and IL cells.

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