Development of the secretory capacity of the chick embryo adrenal glands

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SUMMARY

The secretory capacity of the chick embryo adrenal was investigated at different stages of its development employing a sensitive bioassay for corticosteroids based upon the modifications produced in vitro by these hormones on the height of the chick embryo duodenal mucosa.

The adrenal gland cultivated in contact with explants of duodenum and stimulated with increasing doses of ACTH exhibited a mucosal response similar to that produced by corticosterone, both in the slope of the bioassay dose-response curve and in the histological pattern.

It has been found that the chick embryo adrenal glands can secrete corticoids from the 5th day onwards by adding ACTH to the culture medium. The basal (unstimulated) secretory capacity of the gland (registered from the 8th day on) is significantly correlated with the total protein content of the adrenals in the different stages of development studied.

The maximal secretory capacity of the gland (measured with the dose of ACTH which produces the maximal response) increases steadily up to the 10th day.

The rate of increase of corticosteroid secretion due to ACTH is a constant within the interval of development investigated.

INTRODUCTION

In a previous paper (Pedernera, 1970) the author described a method for the assay of corticosteroids at low concentrations (0.01 µg/ml) based on the effect of these compounds on the chick embryo duodenal mucosa cultivated in vitro. The specificity, sensitivity, precision and advantages of this bioassay have been discussed elsewhere (Pedernera, 1970). This technique has been employed to demonstrate and quantitize in vitro the secretion of corticoids by the chick embryo adrenal gland at different stages of development. The overall information afforded by the experiments herein reported suggests that as soon as the adrenal glands can be recognized they can secrete cortical steroids.

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MATERIALS AND METHODS

The adrenal glands of chick Hy-line embryos of 5, 6, 7, 8, 10, 12, 14 and 16 days of development were carefully dissected. The glands from 12-day or older embryos were decapsulated.

In order to be employed as simultaneous control tissue, metanephros of chick embryos of 10 days of development was dissected and cut into pieces of a size similar to that of the adrenal of the same age.

The adrenal explants and the pieces of metanephros were cultivated in organ culture in contact with duodenum explants (from 16-day-old chick embryos) obtained by cutting the tissue longitudinally and transversely to obtain pieces of 0.5 mm².

In all the experiments the explants were cultivated for 48 h at 37 °C with air as the gas phase. The culture medium was prepared as described previously (Pedernera, 1970).

ACTH (mammalian origin, lyophilized, Actonar, Lab. Acton, Argentina) and corticosterone were added to the medium, when necessary, before agar solidification, to obtain the desired hormonal concentration.

The experimental groups were designed as follows:
(a) Adrenals cultivated in contact with duodenum explants.
(b) Adrenals stimulated with different doses of ACTH and cultivated in contact with duodenum explants.
(c) Duodenum explants cultivated alone.
(d) Duodenum explants cultivated with the addition of different doses of corticosterone in the culture medium.
(e) Metanephros with and without ACTH (1.0 i.u./ml) cultured in contact with duodenum explants.

The first four groups were carried out simultaneously in all cultures.

At the end of the incubation period, the explants were prepared for histological study and the height of the duodenal mucosa was measured as formerly reported (Pedernera, 1970).

The total protein content of chick embryo adrenals from 6 to 16 days of development was estimated by the micro-Kjeldhal technique; ammonia was measured according to Mann (1963).

Standard statistical methods were employed for the evaluation of the data (Snedecor & Cochran, 1967; Bliss, 1952).

RESULTS

The adrenal glands and the duodenum explants cultivated in contact appeared fused at the end of the incubation period. The duodenum explants which had been cultivated with adrenal glands exhibited oedema in the connective tissue of the mucosa and dilated vascular channels in the deepest layer of the mucosa.
Fig. 1. Histological sections of chick embryo duodenum of 16 days of development cultivated for 48 h. (A) Control. (B) Treated with corticosterone (4.0 µg/ml). (C) Cultivated in contact with a chick embryo adrenal gland of 10 days of development treated with ACTH (1.0 i.u./ml).
near the limit of the muscular layer. Similar phenomena were produced by adding corticosterone (Fig. 1).

The changes in the height of the duodenal mucosa cultivated in contact with chick embryo adrenal glands at the 10th day of development are reported in Table 1. The results indicate that the presence of adrenal tissue increases the height of the duodenal mucosa; this effect is magnified by the addition of ACTH to the medium. The presence of metanephros tissue alone or with ACTH does not affect the mucosal height. Explants of duodenum alone were cultured with several doses of ACTH (0.06 to 4.0 i.u./ml) without any effect.

<table>
<thead>
<tr>
<th>Tissue</th>
<th>Controls</th>
<th>ACTH (1.0 i.u./ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Adrenal and duodenum</td>
<td>208.2 ± 4.2 (9)**</td>
<td>236.4 ± 7.0 (13)**</td>
</tr>
<tr>
<td>Duodenum</td>
<td>172.6 ± 4.8 (15)</td>
<td>178.2 ± 3.6 (10)</td>
</tr>
<tr>
<td>Metanephros and duodenum</td>
<td>177.6 ± 5.0 (10)</td>
<td>183.2 ± 3.6 (15)</td>
</tr>
</tbody>
</table>

** Values are expressed as mean ± s.e. (no. of observations). Significantly different from control (P < 0.01).

*** Significantly different from duodenum alone (P < 0.001).

![Graph showing the response of the duodenal mucosa to corticosterone and ACTH](image)

Fig. 2. A typical experiment. Response of the duodenal mucosa (height) to the logarithm of corticosterone concentration (●). Response of the duodenal mucosa cultivated in contact with adrenal glands of 10-day-old chick embryos, stimulated with increasing doses of ACTH (logarithmic scale) (○). Each point represents the mean of 12 explants. Bars indicate s.e.

The response of the chick embryo duodenal mucosa to different doses of corticosterone was determined simultaneously with the response to adrenal glands of 10 days of development stimulated with increasing doses of ACTH.
The two slopes did not differ significantly ($F, 0.21; P > 0.05$). With this experimental design the responses of adrenal glands at different embryonic ages were explored. In all cases the slopes of the ACTH dose–response curves were not significantly different from those of corticosterone.

To compare the hormone production by the adrenals of different ages, the values of the mucosal height were converted into the corresponding corticosterone concentration (µg/ml) which produces the same height-increasing effect on the mucosa.

For each age the effect of non-stimulated adrenals on the duodenal mucosa was measured, transformed into µg/ml of corticosterone equivalents and assumed to represent the basal secretion.

The responses of adrenals from 6 to 16 days of development to increasing doses of ACTH can be seen in Fig. 3. The slopes were not significantly different ($F, 0.02; P > 0.05$). The adrenals of chick embryos of 5 days of development were also investigated with the same technique. The response obtained was at the limit of sensitivity of our method. Using several doses of ACTH no slope could be detected and the estimated secretion value, with 0.12 i.u./ml of ACTH in the culture medium, was 0.04 µg/ml in corticosterone equivalents.

The basal secretion of corticoids increases steadily as a function of age (Fig. 4) from the eighth day on. This is, in consequence, the lowest age where basal secretion could be measured. Within the same age range the adrenal
Fig. 4. Correlation between the logarithm of the estimated secretion of the adrenal glands (in $\mu g/ml$ equivalents of corticosterone) and the age of the embryos. Slopes of basal secretion (○) and secretion stimulated with 0.25 i.u./ml (●) and 1.0 i.u./ml (○) of ACTH are parallel. Each point represents the average of the estimated secretion of 10 adrenal glands.

Fig. 5. Correlation between the mass of adrenal tissue (expressed as $\mu g$ of protein per adrenal gland) and the estimated basal secretion (in ng/ml equivalents of corticosterone). Each point is the mean value of 10 chick embryo adrenals at the same state of development (8–16 days).
glands stimulated with ACTH (0.25 and 1.0 i.u./ml) showed a secretion with slopes at higher levels parallel to the basal ($F$, 1.22; $P > 0.05$).

The total protein content of the adrenal glands between 8 and 16 days of development and their basal corticoid secretion were significantly correlated ($r$, 0.92; $P < 0.05$) (Fig. 5).

By increasing the concentration of ACTH in the culture medium it is possible to explore the maximal response in the dose–response curve of the embryonic adrenals to ACTH. As can be seen in Figs. 3 and 6 the dose of ACTH necessary to produce the maximal response increases up to the 10th day when it reaches its maximum.

![Graph showing doses of ACTH (i.u./ml) in the culture medium necessary to produce the maximal response in chick embryo adrenals at different stages of development.](image)

**DISCUSSION**

The adrenocortical material of the chick embryo, apparently produced by proliferation of coelomic mesothelium medial to the mesonephros, is already delimited by the end of the fourth day of development (Willier, 1930).

A 3-β-ol-dehydrogenase activity has been demonstrated on the 4th day (Ericson & Domm, 1969) and the presence of lipids and cholesterol on the 5th day of development (Dawson, 1953; Castañé Decoud, Pedernera & Narbaitz, 1964). Additional evidence has been reported which clearly indicates that the chick embryo adrenal cortex is functioning from the 12th day of development (Moog & Ford, 1957; Stoll, Faucounau & Maraud, 1964; Bonhommet & Weniger, 1967). It is not known, however, the moment at which the gland acquires its functional state during embryological development.
From the several available methods for exploring adrenal cortex function (histology, histochemistry, biochemistry, etc.), the demonstration of corticoid secretion is the most reliable and has been largely employed. Bonhommet & Weniger (1967) used this method for the study of the adrenal cortex secretion in 14-day-old chick embryos. The use of an endocrine gland maintained in organ culture in contact with a target tissue was suggested by Price, Ortiz & Zaaijer (1963), who employed testis and prostate gland.

The duodenum of the chick embryo seems to be a target tissue for biologically active corticosteroids (corticosterone, aldosterone, cortisol and DOC). Other compounds such as oestradiol, testosterone, 17-hydroxy 11-desoxy-corticosterone, adrenalin and noradrenalin do not increase the height of the duodenal mucosa. This is, moreover, a sensitive target tissue because it responds significantly to a corticosterone concentration of 0.015 μg/ml. The precision index of the bioassay is satisfactory (λ, 0.22) (Pedernera, 1970). This method uses the same equipment and technology employed in a laboratory of tissue culture. In culture, the adrenal–duodenum explants fuse to form a single explant (Fig. 1) which ensures that the adrenal secretion reaches the target tissue.

As the response of the duodenum presumably is a function of the corticoid concentration and the time of culture, the method employed estimates the concentration of corticosteroids in the medium necessary to produce a similar effect to that produced by a known concentration of corticosterone used as standard.

The comparison of the slopes of the dose–response curves obtained for duodenal mucosa with different doses of corticosterone and with adrenals stimulated with increasing doses of ACTH showed that they were parallel. This has been taken as evidence for the secretion of corticoids by the adrenals. Additional evidence was provided by the similarities in the histological pictures.

The secretion of corticoids had been demonstrated for the chick embryo adrenal at the 14th day of development (Bonhommet & Weniger, 1967). With the present method the hormonal secretion could be detected from the 8th day on and from the 5th day on by adding ACTH to the medium. That the chick embryo adrenal responds to ACTH from the 5th day has already been shown both in vivo (Castañé Decoud et al. 1964) and in vitro (Pedernera, 1968).

A significant correlation was obtained between the basal secretion and the adrenal mass estimated by its protein content. This has been interpreted to mean that the increase in basal secretion can be attributed to the increase in the adrenal mass during development.

The dose of ACTH necessary to produce the maximal response increases up to the 10th day of development (Fig. 6). This finding seems to indicate that chick embryo adrenals at the 6th day of development have mature enzymic systems as shown by their response to ACTH; however, the concentration of the
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enzymic systems within the cells necessary for the synthesis of corticoids increases up to the 10th day.

As the regression line of basal secretion on age parallels that of the secretion due to ACTH (Fig. 4) the inference is made that the sensitivity to ACTH remains constant in the period investigated.

Résumé

Le développement de la capacité sécrétoire des surrénales de l'embryon de Poulet

La capacité sécrétoire de la surrenale de l'embryon de poulet a été recherchée dans les différents états de son développement en employant un essai biologique pour les corticoides, basé sur l'effet de ces hormones sur la hauteur de la muqueuse duodénale de l'embryon de poulet.

Les surrénales cultivées en contact avec le duodenum et stimulées avec des doses différentes de ACTH, déterminent un effet sur la muqueuse duodénale semblable à celui produit par la corticostérone, soit dans l'image histologique, soit la courbe de dose-réponse de l'essai.

Les surrénales d'embryon de poulet de cinq jours peuvent secrétérer des corticoides en ajoutant ACTH à la culture. La sécrétion base (non stimulée) des surrénales est enregistrée à partir du huitième jour du développement et correlationnée significativement avec le contenu de protéine de la glande dans les différentes étapes du développement que l'on a étudié.

La capacité de sécrétion maximum de la glande (mesurée comme la dose de ACTH qui produit la majeure réponse) augmente jusqu'au dixième jour de son développement.

Dans cette étude la taxe d'augmentation de la sécrétion de corticoides surrénaïens produit par l'hormone corticotrope est une constante.

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REFERENCES


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