

HYPOPHYSEAL CONTROL OF CORTICOSTERONE PRODUCTION IN TWO ZONES OF THE ADRENAL IN COCKERELS. IN VITRO STUDIES

by

L. KONDICS

Department of General Zoology of the Eötvös Loránd University, Budapest

Received on 16th June 1970

It is a well-known fact that in the adrenal gland of mammals mineral corticoids are produced by the zona glomerulosa, and glucocorticoids mainly by the zona fasciculata and reticularis (Deane et al., 1948, Farrel et al., 1956, Goldman - Ronzoni 1956, Hartroft - Eisenstein 1957, Stachenko - Giroud 1959/a, 1959/b). The structure of the adrenal cortex of avians differs from that of mammals. However, in certain cases the interrenal cells producing steroids also form two zones resembling those found in mammals (Arvy 1961, Bhattacharyya - Ghosh 1963, Burger 1938, Ghosh 1963, Knouff - Hartman 1951, Lorenzen - Farnier 1964, Miller - Riddle 1942, Miller 1961, Sauer - Latimer 1931, Sinha - Ghosh 1961, 1964). It has been known for a comparatively long time that the two main hormones of the avian adrenal cortex are aldosterone and corticosterone (De Roos 1961, 1964, 1969), their localization, however, is unknown. In domestic pigeons it has been shown that the outer subcapsular zone of the adrenal cortex undergoes atrophy due to continuous administration of NaCl. From the foregoing it was concluded that in avians the outer zone, similarly to the zona glomerulosa in mammals, produces aldosterone and the more deeply situated wider zone, analogous to the zona fasciculata, produces corticosterone (Kondics 1963). In the present study data obtained by "in vitro" methods on the corticosterone production of the two zones of the adrenal cortex in cockerels, further the hypophyseal control of these zones are presented.

Material and Method

Ten 11-month old white leghorn cockerels were decapitated and the adrenal glands were cut into 1 mm thick discs with a device made of razor blades. Under a binocular microscope 15-20 mg sections were prepared of the inner and outer zones in Krebs-Ringer solution with the

help of a fine knife made from a splintered razor blade. The specimens taken from the two zones were incubated in the usual way, after pre-incubation of 15 minutes in Krebs-Ringer buffer solution, for an hour with a constant perfusion of a mixture of 95% oxygen and 5% carbon dioxide. The corticosteron produced was determined by fluorimetry (Silber et al., 1958). The experiment was later repeated with the modification that $3 \times 15 - 20$ mg pieces were taken from both zones and at the beginning of the incubation 0,05 and 0,15 IU ACTH/100 mg adrenal was added to 2 specimens, respectively.

Results

The histological picture of the cockerel adrenal cortex can be seen in Fig. 1. The adrenal cells producing catecholamines form isles between the interrenal cells secreting steroids. In the outer zone more interrenal cells can be seen in the microscopic picture than in the picture of the inner zone. The fresh cells of the peripheral zone are lemon-yellow in adult cockerels, while those of the inner zone are white, therefore the two zones can be readily separated during preparation.

In Fig. 2 the corticosterone production of the inner and outer zones is shown. In the untreated animals the peripheral zone produces much more corticosterone, as compared to the outer zone.

In Fig. 3 the secretional activity of the zones is shown after the addition of ACTH. The treatment stimulated the activity of both zones, however, the increase in corticosterone production was more significant in the inner zone, so that 0,15 IU ACTH/100 mg adrenal sufficed for the inner zone to exceed the production of the outer zone.

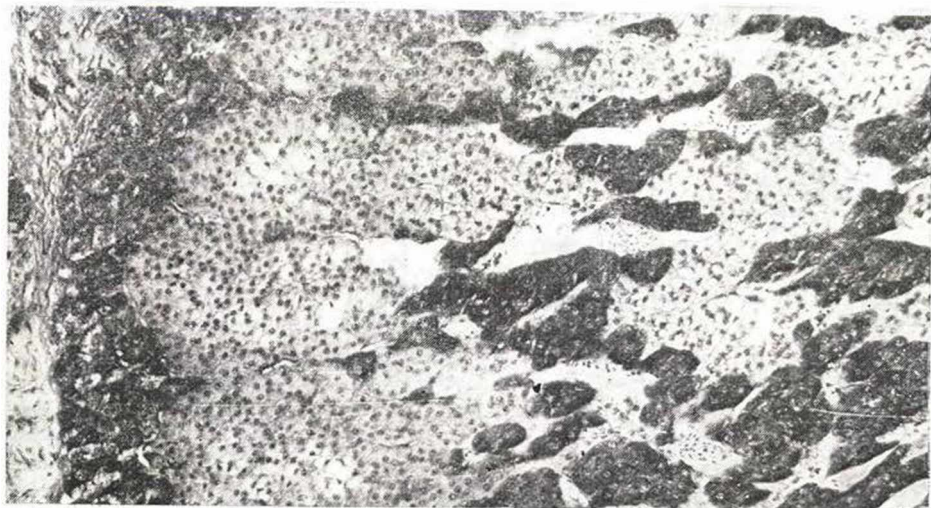


Fig. 1.

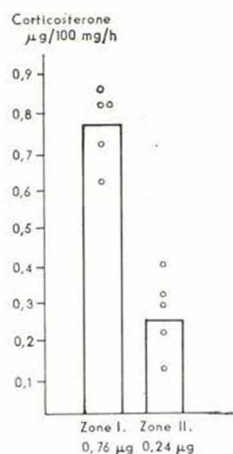


Fig. 2.

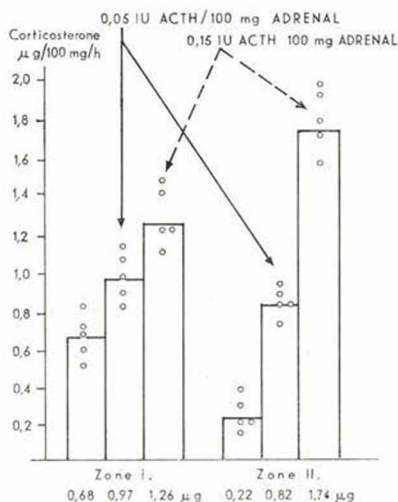


Fig. 3.

Discussion

Our own studies (Kondics 1963, 1964, 1965, 1968), as well as the results of other authors (Bhattacharya — Ghosh 1963, Lorenzen — Farner 1964, Miller — Riddle 1942, Sinha — Ghosh 1964) have proved that each zone of the avian adrenal gland reacts to the different stresses affecting the organism in different ways, even in cases where, under controlled circumstances, no morphological difference exist between the two zones. The difference in the response between the two zones of the avian adrenal cortex can probably be explained by the difference in the localization of aldosterone and corticosterone. We have shown that, by administering food rich in salt, the peripherally situated interrenal cells of the adrenal gland become selectively atrophied. From this phenomenon we have concluded that in avians, similarly to mammals, aldosterone is most probably localized in the peripheral zone, and that corticosterone is produced in the inner zone (Kondics 1963).

The corticosterone production of the peripheral and inner zone of the cockerel seems to be in contrast with the above suggestion. Planimetric measurements carried out to gain general information, showed that in the peripheral zone of cockerels the number of interrenal cells exceeded by a factor of 2 to 3 the number of similar cells found in the inner zone. Consequently, the peripheral zone produces 2–3 times more corticosterone, which naturally accounts for the fact that in the control animals the greater part of corticosterone was produced by the peripheral

zone. This, however is not in contrast with the supposed presence of aldosterone, since it is known that corticosterone is the precursor of aldosterone. However, the high glucocorticoid production is surprising, since glucocorticoid is produced in the peripheral zone of the mammal adrenal glands in small quantities, only (Stachenko - Giroud 1959a,b). In this respect the difference between avian and mammal adrenal glands is significant.

Due to the effect of ACTH the corticosterone production of both zones increases, however, in the inner zone to a larger extent and thus the administration of 0,15 IU ACTH/100 mg adrenal suffices for the inner zone to surpass significantly the production of the peripheral zone. This means that the ACTH response of the two zones is different: the outer zone secretes a significant amount of corticosterone at low ACTH level, while the inner zone can only do that at a higher level of ACTH secretion. The slower increase of corticosterone production in the outer zone in the case of stimulation might be explained by the increased corticosterone aldosterone transformation. It is a well-known fact that in avians ACTH intensifies aldosterone secretion (De Roos 1969).

The above statements are all in agreement with the results of other authors and also with our own findings obtained in experiments carried out with pigeons. It was shown that by stimulating the adrenal gland, growth in nuclear volume increased in the inner zone and, parallelly, the rate of decrease in sudanophilia was also faster. If endogenous ACTH is suppressed by prednisolone, atrophy will only affect the inner zone, the peripheral zone, on the other hand, will remain unchanged (Kondics 1965). Accordingly, atrophy will be restricted to the inner zone after hypophysectomy (Miller - Riddle 1942, Miller 1961).

On the basis of the above results, the question of the autonomy of avian adrenal glands can be raised. It is known that the decrease in the weight of the adrenal gland after hypophysectomy, in contrast to other endocrine organs, is significantly less in avians (Schoolley et al. 1941). On the other hand, steroid production decreases only to 37 per cent of the original value, at the most (Brown 1961, Frankel et al. 1967). These findings suggest that avian adrenal glands are, to a certain degree, autonomous, i.e. the adrenal gland is able to perform certain basic secretory activities without the participation of the pituitary gland. However, if exposed to stress, the adrenal gland of a hypophysectomized animal is not able to increase its activity (Brown 1961). Others explain the relatively high basic secretion without the help of ACTH by the extrahypophyseal stimulative factor, i.e. with extrahypophyseal ACTH, which can be localized in the eminentia mediana (Péczeley - Zboray 1967, Resko et al. 1964). In our opinion the idea of the relative autonomy of the adrenal gland seems more acceptable, mainly due to the arguments brought forward in the present paper and based on the lack of relative ACTH response of the peripheral zone.

Summary

1. Under controlled conditions, the peripheral zone of the adrenal cortex in cockerels secretes approx. 3 times more corticosterone, as compared to the inner zone. This is due first of all to the greater number of interrenal cells.

2. Under the effect of ACTH, the corticosterone production of both zones increases, however growth in the inner zone is faster. Upon the administration of 0,15 IU ACTH/100 mg adrenal, the production of the inner zone will significantly surpass that of the peripheral zone. The explanation of the above phenomenon is to be sought in the difference between the sensitivity of the two zones to ACTH and in the increased corticosterone aldosterone transformation in the peripheral zone.

REFERENCES

- Arvy, L. 1961. Contribution à l'histochimie de la glande surrénale chez *Gallus domesticus* L. et chez *Anas boschas* L. *Compt. Rend. Soc. Biol.* **155**: 69–71.
- Bhattacharyya, T. K. — Ghosh, A. 1963. Histological and histochemical studies of the adrenal cortex in experimentally hypothyroid pigeons. *Acta Anat.* **52**: 150–162.
- Brown, K. J. 1961. The validity of using plasma corticosterone as a measure of stress in turkey, *Proc. Soc. exp. Biol. Med.* **107**: 538–542.
- Burger, J. W. 1938. Cyclic changes in the thyroid and adrenal cortex of the male starling, *Sturnus vulgaris*, and their relation to the sexual cycle. *Am. Naturalist* **72**: 562–570.
- Deane, H. W. — Shaw, J. H. — Greep, R. O. The effect of altered sodium or potassium intake on the width and cytochemistry of the zona glomerulosa of the rat's adrenal cortex. *Endocrinology* **43**: 133–153.
- DeRoo's, R. 1961. The corticoids of the avian adrenal gland. *Gen. Comp. Endocrinol.* **1**: 494–512.
- DeRoo's, R., — DeRoo's, C. 1964. Effects of mammalian corticotropin and chicken adenohipophysial extracts on steroidogenesis by chicken adrenal tissue in vitro. *Gen. Comp. Endocrinol.* **4**: 602–607.
- DeRoo's, R. 1969. Effects of mammalian corticotropin and progesteron on corticoid production by chicken adrenal tissue in vitro. *Gen. Comp. Endocrinol.* **13**: 455–459.
- Farrel, G. L. — Banks, R. C. — Koletsky, S. 1956. The effect of corticosteroid injection on aldosteron secretion. *Endocrinology* **58**: 104–108.
- Frankel, A. F. — Graber, J. W. — Nalbandov, A. V. 1967. Adrenal function in cockerels. *Endocrinology* **80**: 1013–1019.
- Ghosh, A. 1962. A comparative study of the histochemistry of the avian adrenals. *Gen. Comp. Endocrinol. Suppl.* **1**: 75–80.
- Goldman, M. L. — Ronzoni, H. A. 1956. The response of the adrenal cortex of the rat to dietary salt restriction and replacement. *Endocrinology* **58**: 57–61.
- Hartroft, P. M. — Eisenstein, A. B. 1957. Alterations in the adrenal cortex of the rat induced by sodium deficiency: correlation of histological changes with steroid hormone secretion. *Endocrinology* **60**: 641–651.
- Kondics, L. 1963. Über die Wirkung des Kochsalzes auf die interrenalen Zellen der Nebenniere bei Haustauben. *Ann Univ. Sci. Budapest. Sect. Biol.* **6**: 101–107.

- Kondics, L. 1964. Über die Wirkung der Dehydration auf die funktionelle Zonation der Nebenniere bei Haustauben. *Ann. Univ. Sci. Budapest, Sect. Biol.* **7**: 115–120.
- Kondics, L. 1965. Die Wirkung von ACTH und Prednisolon auf die funktionelle Zonation der Nebenniere bei der Taube. *Acta Morph. Acad. Sci. Hung. Budapest*, **13**: 233–240.
- Kondics, L. 1968. Durch fortdauernde Wasserbelastung bewirkte Atrophie der interrenalren Zellen der Taube-Nebenniere. *Annal. Univ. Sci. Budapest, Sect. Biol.* **9**: 227–230.
- Knouff, R. A. — Hartman, F. A. 1951. A microscopic study of the adrenal of the brown pelican. *Anat. Record* **109**: 161–187.
- Lorenzen, L. C. — Farnier, D. S. 1964. An annual cycle in the interrenal tissue of the adrenal gland of the white crowned sparrow, *Zonotrichia leucophrys gambellii*. *Gen. Comp. Endocrinol.* **4**: 253–263.
- Miller, R. A. — Riddle, O. 1942. The cytology of the adrenal cortex of normal pigeons and in experimentally induced atrophy and hypertrophy. *Am. J. Anat.* **71**: 311–335.
- Miller, R. A. 1961. Hypertrophic adrenals and their response to stress after lesion in the medium eminence of totally hypophysectomized pigeons. *Acta Endocrinol.* **37**: 565–576.
- Péczeley, P. — Zboray, G. 1967. CFR and ACTH activity in the medium eminence of the pigeon. *Acta Physiol. Acad. Sci. Hung.* **32**: 229–239.
- Resko, J. A. — Norton, H. W. — Nalbandov, A. V. 1964. Endocrine control of the adrenal in chickens. *Endocrinology* **75**: 192–200.
- Sauer, F. C. — Latimer, H. B. 1931. Sex differences in the proportion of the cortex and the medulla in the chicken suprarenal. *Anat. Record* **50**: 285–298.
- Schooley, J. P. — Riddle, O. — Bates, R. W. 1941. Replacement therapy in hypophysectomized juvenile pigeons. *Am. J. Anat.* **69**: 123–154.
- Silber, R. H. — Bush, R. D. — Oslaps, R. 1958. Practical procedure for estimation of corticosterone or hydrocortison. *Clin. Chem.* **4**: 278–285.
- Sinha, D. — Ghosh, A. 1961. Some aspects of adrenocortical cytochemistry in the domestic pigeon. *Endocrinologie* **40**: 270–280.
- Sinha, D. — Ghosh, A. 1964. Cytochemical study of the suprarenal cortex of the pigeon under altered electrolytic balance. *Acta Histochem.* **77**: 222–229.
- Stachenko, J. — Giroud, J. P. 1959. a. Functional zonation of the adrenal cortex. Pathways of corticosteroid biogenesis. *Endocrinology* **64**: 730–742.
- Stachenko, J. — Giroud, J. P. 1959b. Functional zonation of the adrenal cortex: site of ACTH action. *Endocrinology* **64**: 743–752.