Int J Endocrinol Metab 2005; 2:93-98

ORIGINAL ARTICLE

Age-Related Changes in the In Vivo Adrenocortical Production of cAMP and Corticosterone in Response to Exogenous ACTH in Long-Evans Female Rats

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Ageing effects on the in vivo cyclic 3,5'-adenosine monophosphate (cAMP) production by the adrenal cortex were studied in the rat.

Materials and Methods: Eleven old (from 23 to 29 months) and 13 young (from 4 to 5 months), dexamethasone pre-treated Long-Evans female rats received 5.0 mu.i. (1-24) ACTH/100g b.w. by intravenous injection. The plasma concentration of corticosterone as well as the adrenal contents in cAMP and corticosterone were measured, by radioimmunoassay, just before and 45 min after the (1-24) ACTH injection.

Results: The basal plasma corticosterone level and the adrenal contents in corticosterone and cAMP were low and no group difference was observed. The (1-24) ACTH injection causes significant increases in the plasma corticosterone level and the glandular contents in corticosterone and cAMP, which were lesser in the old animals than in the young ones; the differences aged/young were approximately -37%, -18% and -55% respectively.

Conclusions: These results suggest that the reduced steroïdogene response of the adrenal cortex in the old rat, to an acute ACTH administration, is at least partly due to a decrease in the cellular production of the principal second messenger of this hormone, i.e. the cAMP.

Key words: adrenal cortex, (1-24) ACTH, corticosterone, cAMP, dexamethasone, Long-Evans, female rat

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Introduction

An age-related decline in the adrenal stimulated secretion of corticosterone was documented by a number of studies carried out in the rat in vivo[1-5] as well as in vitro.[7-13] We have previously proposed that this impairment might be in part due to a reduced production of the adrenocorticotropin hormone (ACTH) in response to the corticotropin releasing hormone (CRH).[14] We have also observed that the in vivo capacity of the adrenal cortex to produce corticosterone and aldosterone, in response to ACTH, was significantly reduced in old Long-Evans female rats.[15] In adult adrenal cells, the interaction between ACTH and its adrenal receptors is followed by the activation of adenylate cyclase system, via a G protein, which leads to an increased production of cyclic 3,5'-

adenosine monophosphate (cAMP).[16] In turn, the cAMP actives a protein kinase A which induces the phosphorylation of many proteins inducing biological responses such as corticosteroid biosynthesis.[17] There is presently only limited information about the intracellular mechanisms of ACTH action on the adrenal cell during ageing particularly cAMP production. We did not find any study on the in vivo production of cAMP by adrenal cells in ageing animals, and some in vitro studies, carried out in the rat led to opposite conclusions.[7,9,12,13] In addition, insufficiently old animals were used in these studies. In the present work, the in vivo corticosterone and cAMP production by the adrenal cortex in response to ACTH injected intravenously was studied in female rats, which were sufficiently senescent, and dexamethasone pretreated.

Materials and Methods

Eleven aged (23 to 29 months) and 13 young (4 to 5 months) female Long-Evans rats were used in his study. They were bred under conventional conditions in our animal care facility and housed individually at least 8 days before the experimentation. Food and water tap were provided ad libitum. All experiments started at 8.30h. In order to avoid a possible effect of endogenous ACTH levels; each rat was pre-treated with dexamethasone (Dectancy ®, Roussel, Paris, France) dissolved in drinking water 20 hours before the experimentation (170µg/100g b.w.). The rats were anaesthetized by an injection (1 ml/100g i.p.) of a solution containing 5.0 mg of pentobarbital (Clin-Midy; Paris, France) and 2.5 µg of atropine (Meram; Melun, France). Fifteen minutes after anaesthesia, a local anaesthesia was practised by subcutaneous microinjections of 2% xylcaïne (Bellon; Neuilly-sur-seine, France), an incision (1.5 cm) in cutaneous and muscular plans of the back was carried out and the left adrenal, which was quickly removed after having ligatured its blood vessels, to be used to determine the basal corticosterone and cAMP contents. Local anaesthesia was again given at the level of the neck near the left clavicle. Here, an incision (1 cm) of the skin and muscles make it possible to expose the subclavian sinus venous in which a fine needle connected to a heparinized polyethylene catheter was inserted. This device was used both to collect blood samples and to inject ACTH (Synacthene®, Ciba, Rueil-Malmaison, France). Thirty minutes after anaesthesia, a zero time blood sample (1 ml) was slowly (in two minutes) removed. The sampling was immediately followed by an injection of 5.0 mu.i. ACTH (1-24)/100 g b.w. (in 0.1 ml of saline with 0.5% of BSA). The animals were sacrificed by decapitation 45 min thereafter (time of the maximum adrenal response to ACTH), the right adrenal was removed and blood, collected in calcium heparinate solution, was centrifuged. The plasma intended for corticosterone assays, was stored in –30°C. Straight after they were taken, the adrenals were degreased on a cooled glass plate, and then weighed. A small incision of the adrenal capsule and a light pressure on the gland with grips, allow separating the capsule, to which adhere cells of the zona glomerulosa, and the remainder of the adrenal. The two adrenals parts were separately weighed and stored at -80°C. Assays of cAMP, corticosterone and total proteins were performed in the free adrenal gland capsule. The decapsuled adrenals were cut out, and then separately ground in distilled water (4 ml) with a teflon-glass homogeniser. Three ml of cold ethanol were added to 0.8 ml of homogenate and after one hour stay at -30°C, for the proteins precipitation, the mixture was centrifuged during 20 min with 4000 rpm at 4°C. Then the supernatant was dried by evaporation. The cAMP concentrations were measured using a cAMP radioimmunoassay kit (Immunotech International, ref.1117). The sensitivity of the assay was 0.2 nM, the inter- and intra-assay variations were 6.7% and 9.0% respectively.

The corticosterone concentrations were determined, after dichloromethane extraction,

by a competitive protein-binding radioassay using plasma (2%) from adrenalectomized female rats as the source of corticosteroid-binding globulin. The inter- and intra-assay variations were 6.2% and 8.5% respectively.

The protein content was determined by the Bradford microassay technique using BSA as standard.

Data were expressed as means ± S.E.M). The difference between two mean values was evaluated using (Fisher) Student's t test.

Results

The mean body weight and the mean weight of the right and left adrenals (table I) were higher in the aged animals than in the younger ones (respectively +29.5%, $p<0.001$, and +10.5%, $p<0.05$). During the 20 hours of pretreatment to the dexamethasone, the old rats consumed a little more water than the young rats (+11%, $p<0.05$).

Expressed in µg/100 ml (Fig.1), the basal corticosteronemia of the old female rats (1.9 ±0.5) was low and not significantly different from that of the young rats (1.2± 0.3). The ACTH injection leads to an increased production of corticosterone in the two age groups, but the response of the aged rats (31.3±2.0) was significantly lower ($p<0.001$) than that of the young ones (49.8±2.8)

The adrenal corticosterone content (Fig.2), expressed by ng/mg in the left adrenal before ACTH injection was weak and no difference was observed between old (1.3±0.1) and young (1.0±0.1) female rats. ACTH significantly increased the adrenal hormone contents in both age groups. However, the values observed were significantly lower ($p<0.05$) in the old (58.4±3.2) than in the young rats (71.3±4.8).

The concentrations in cAMP (Fig.3 a and b) of the left adrenal not stimulated by (1-24) ACTH were, when expressed in pmol by mg of gland or mg of proteins, respectively 1.5±0,2 and 16.6±3.0 in the young animal and 1.1±0.1 and 12.6±2.2 in the old female rats. The differences old/young were respectively –26.7% ($p<0.2$) and –24.1 % ($p<0.3$). Forty five min after the injection of 5.0 mu.i. (1-24) ACTH, the content of the right adrenal was significantly increased. In the young female rats, it increased to 11.4±1.4 pmol/mg or 129.3±16,1 pmol/mg of proteins. In the old

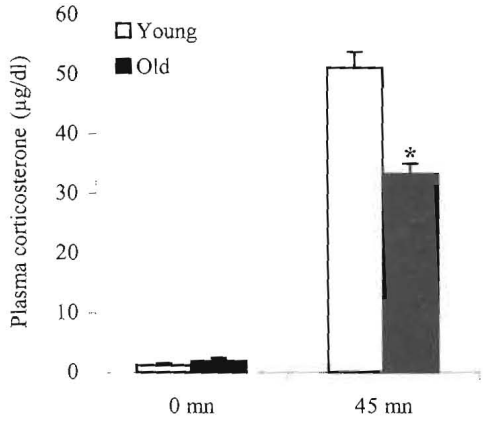

Fig.1. Effect of 5 mu.i. (1-24) ACTH/100 g b.w. on the plasma corticosterone concentration in young and old dexamethasone pre-treated female Long-Evans rats. *p<0.001.

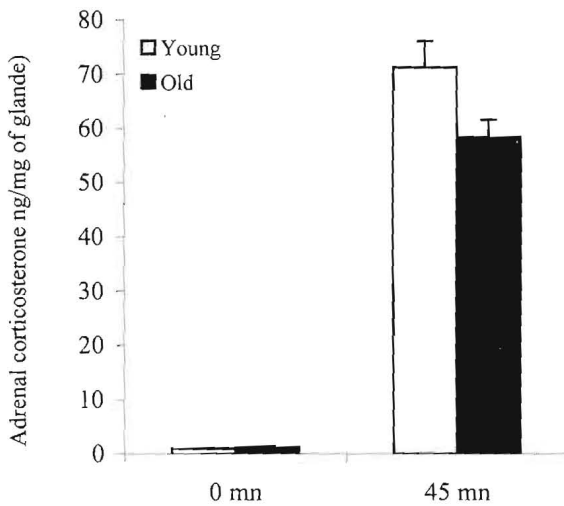

Fig.2. Effect of 5 mu.i. (1-24) ACTH/100 g b.w. on the adrenal corticosterone content in young and old dexamethasone pre-treated female Long-Evans rats. P<0.05.

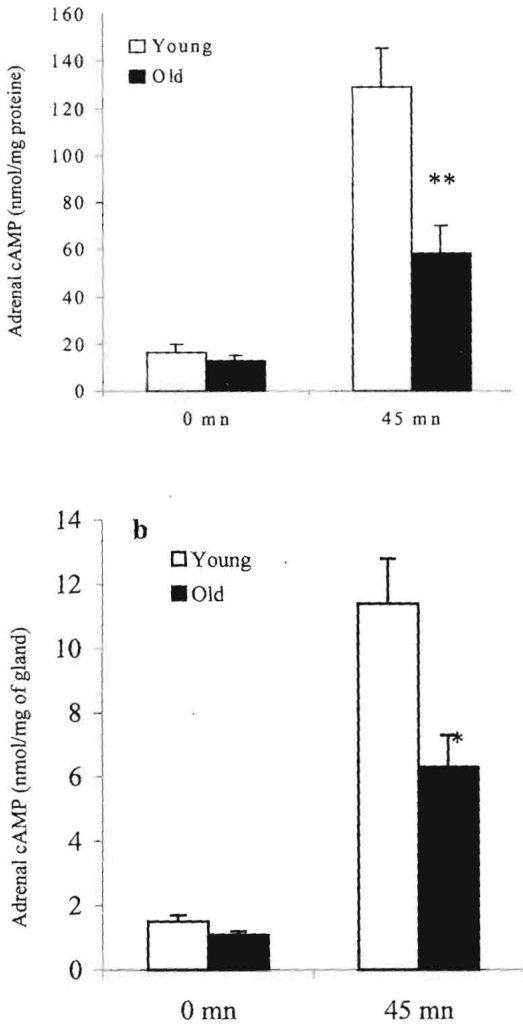

Fig.3. Effect of 5 mu.i. (1-24) ACTH/100 g b.w. on the adrenal cAMP content (a: in nmol/mg protein, b: in nmol/mg of adrenal gland) in young and old dexamethasone pre-treated female Long-Evans rats. ** p<0.01, * p<0.05

female rats, the glandular concentrations of cAMP were then 6.3±1.0 pmol/mg or 58.2±11.6 pmol/mg protein and were lower than that of the youngest. The differences old/young of the concentrations were -5.1 pmol/mg (-44.7%) or -71.1 pmol/mg protein (-55.0%) and were statistically significant ($p<0.01$ and $p<0.003$).

Discussion

In response to an acute injection of 5.0 mu.i. ACTH(1-24)/100g b.w., a lower raise in corticosterone plasma and adrenal concentrations and in the glandular cAMP concentration was observed in the old female Long-Evans rats, compared to the young ones (with a difference of –37%, –18% and –55% respectively). The variations of corticosterone and cAMPc concentrations observed in our animals in response to the ACTH stimulation, reflect probably similar variations in their production levels. Indeed, concentrations of those substances were measured immediately after a single injection of exogenous ACTH, the endogenous production of ACTH being prevented by the dexamethasone pretreatment as proven by the weak basal corticosterone concentrations. Nevertheless, the age effect on the metabolic clearance of injected ACTH and released corticosterone must be precisely determined. Concerning the corticosterone, these results confirm our previous data[15] establishing a significant age-related attenuation in the adrenal response to 0.05 and 5 mu.i. ACTH(1-24)/100g b.w. in both male and female rats. This in vivo observation is in agreement with previous reports[1,2,4,5] but it does not agree with others[18,19] that did not find any significant change in this parameter with age. This attenuation in the adrenal response to ACTH could be due to a deterioration of one or several of the biochemical stages implied in the intracellular mechanisms of the adrenocorticotropin hormone action.

The results of the majority of the studies on the in vitro corticosterone production in response to ACTH, apart those by Scaccianos et al[20] and of Lo et al.[12] give evidence to support the above assumption. Thus, the production of corticosterone by adrenal fragments[7] or by isolated[8] dispersed[9,20] and in culture adrenal

Table 1. Ponderal and metabolic characteristics of young and aged animals used to evaluate the effects of the ACTH on corticosterone and cAMP adrenal concentrations.

	Young	Aged
Body weight (g)	243 ± 5	345 ± 20*
Volume of water drunk (mL/24h)	16.2 ± 1.3	18.0 ± 1.3
Adrenal weight entire left	24.9 ± 1.0	27.8 ± 1.8
Adrenal weight entire right	21.2 ± 0.7	23.7 ± 1.8
Adrenal weight Dec left	18.6 ± 0.7	20.0 ± 1.4
Adrenal weight Dec. right	14.1 ± 0.6	16.1 ± 1.4

* $p<0.00$; Dec= Decapsulated

cells[10] decreases with age in the rat. To our knowledge, no previous study has been published concerning the in vivo production of cAMP induced by ACTH in the adrenal cortex of the old rats and very few investigations were devoted to the effects of ageing on the cellular and molecular mechanisms implied in the corticosteroidogenesis. Nevertheless, some contradictory observations have resulted from in vitro studies using rat adrenal cells. Thus Popplewel et al.[9] consider that the adrenal deficiency observed in 18 months old male Sprague-Dawley rats, in response to ACTH, was due neither to a reduced cAMP production nor to a reduction in the number or sensitivity of the ACTH receptors as suggested by Malamed and Garcia.[8] According to the findings of Popplewel et al,[21] and Popplewel and Azhar[22] ????this deficiency would relate to certain post with second messenger events; precisely the capacity of the cortical cells to collect, synthesize and transform cholesterol, precursor of the steroidogenesis. Neither the contents nor the activity of the microcosmical or mitochondria enzymes undergoes important modifications with age.[21] The studies of Lo et al. lead to other conclusions: reduced corticosterone and cAMP productions by the zona fasciculata cells stimulated by ACTH were observed in 22-23 months old male rats,[20] but an increase in these parameters was reported in middle aged female rats.[12] According to these same authors, this difference in response to ACTH between old male and female rats would be associated with a sex related-modification of the stimulatory effect of prolactin. Unfortunately, these interesting studies of Popplewell and Lo aimed to compare the response of adrenal cells in young rats and in insufficiently old animals (some of them being only 12 months old). Hence, in our opinion, these studies dealt with maturation and not ageing, and they cannot be extrapolated to our study, in which the animals were much older.

In conclusion, our results show clearly that one of the intracellular causes of the adrenal deficiency observed in the old Long-Evans female rat in response to ACTH is the reduced production of the principal second messenger, the cAMP. This observation brings to mind those concerning certain nervous structures where the adenylate cyclase activity and the cAMP generation were reduced during ageing.[23.24] This age-reduced cAMP production is completely compatible with the reported alterations in the cholesterol metabolism,[21,22] i.e. the first stage common to any steroid biosynthesis, and it agrees with our previous results[14,15] which established that the adrenal deficiency accompanying ageing affects simultaneously the corticosterone and aldosterone production.

Acknowledgements

We wish to thank Dr. Orsini J.C. and Dr. Himmi T. for reading the manuscript.

References

1. Hess GD, Riegle GD. Adrenocortical responsiveness to stress and ACTH in aging rats. J Gerontol. 1970t;25(4):354-8.
2. Hess GD, Riegle GD. Effects of chronic ACTH stimulation on adrenocortical function in young and aged rats. Am J Physiol. 1972;222(6):1458-61.
3. Hylka VW, Sonntag WE, Meites J. Reduced ability of old male rats to release ACTH and corticosterone in response to CRF administration. Proc Soc Exp Biol Med. 1984;175(1):1-4.
4. 4 Reaven E, Kostrna M, Ramachandran J, Azhar S. Structure and function changes in rat adrenal glands during aging. Am J Physiol. 1988;255(6 Pt 1):E903-11.
5. Cizza G, Calogero AE, Brady LS, Bagdy G, Bergamini E, Blackman MR, et al. Male Fischer 344/N rats show a progressive central impairment of the hypothalamic-pituitary-adrenal axis with advancing age. Endocrinology. 1994;134(4):1611-20.
6. Hauger RL, Thrivikraman KV, Plotsky PM. Age-related alterations of hypothalamic-pituitary-adrenal axis function in male Fischer 344 rats. Endocrinology. 1994;134(3):1528-36.
7. Pritchett JF, Sartin JL, Marple DN, Harper WL, Till ML. Interaction of aging with in vitro adrenocortical responsiveness to ACTH and cyclic AMP. Horm Res. 1979;10(2-3):96-103.
8. Malamed S, Carsia RV. Aging of the rat adrenocortical cell: response to ACTH and cyclic AMP in vitro. J Gerontol. 1983;38(2):130-6.
9. Popplewell PY, Tsubokawa M, Ramachandran J, Azhar S. Differential effects of aging on adrenocorticotropin receptors, adenosine 3'5'-monophosphate response, and corticosterone secretion in adrenocortical cells from Sprague-Dawley rats. Endocrinology. 1986;119(5):2206-13.
10. Cheng B, Horst IA, Mader SL, Kowal J. Diminished adrenal steroidogenic activity in aging rats: new evidence from adrenal cells cultured from young and aged normal and hypoxic animals. Mol Cell Endocrinol. 1990;73(1):R7-12.
11. Belloni AS, Rebuffat P, Malendowicz LK, Mazzocchi G, Rocco S, Nussdorfer GG. Age-related changes in the morphology and function of the zona glomerulosa of the rat adrenal cortex. Tissue Cell. 1992;24(6):835-42.
12. Lo MJ, Kau MM, Chen JJ, Yeh JY, Lin H, Wang SW, et al. Age-related differences in corticosterone secretion in female rats. Metabolism. 1999;48(4):535-41.
13. Lo MJ, Kau MM, Cho WL, Wang PS. Aging effects on the secretion of corticosterone in male rats. J Investig Med. 2000;48(5):335-42.
14. Brudieux R, Ait Chaoui A, Rakotondrazafy J. Age-related decreases in plasma adrenocorticotropic hormone, corticosterone, and aldosterone responses to exogenous corticotropin-releasing hormone in the rat. Gerontology. 1995;41(6):308-14.
15. Ait-Chaoui A, Rakotondrazafy J, Brudieux R. Age-related changes in plasma corticosterone and aldosterone responses to exogenous ACTH in the rat. Horm Res. 1995;43(5):181-7.
16. Spiegel, A.M., Carter, A., Brann, M., Collins, R., Goldsmith, P., Simonds, W., Vintsky, R., Eide, B., Rossiter, K., Weinstein, L. and Woodard, C. (1988). Signal transduction by arginine nucleotide-binding proteins. Rec. Prog. Horm. Res., 44, 337-371.
17. Saez JM, Morera AM, Dazord A. Mediators of the effects of ACTH on adrenal cells. Adv Cyclic Nucleotide Res. 1981;14:563-79.
18. Sonntag WE, Goliszek AG, Brodish A, Eldridge JC. Diminished diurnal secretion of adrenocorticotropin (ACTH), but not corticosterone, in old male rats: possible relation to increased adrenal sensitivity to ACTH in vivo. Endocrinology. 1987;120(6):2308-15.
19. Meaney MJ, Aitken DH, Sharma S, Viau V. Basal ACTH, corticosterone and corticosterone-binding globulin levels over the diurnal cycle, and age-related changes in hippocampal type I and type II corticosteroid receptor binding capacity in young and aged, handled and nonhandled rats. Neuroendocrinology. 1992;55(2):204-13.
20. Scaccianos, S., Desciullo, A. and Angelucci, L. (1990). Age relates changes in hypothalamo-pituitary-adrenocortical axis activity in the rat. Neuroendocrinology, 52, 150-155.
21. Popplewell PY, Azhar S. Effects of aging on cholesterol content and cholesterol-metabolizing enzymes in the rat adrenal gland. Endocrinology. 1987;121(1):64-73.
22. Popplewell PY, Butte J, Azhar S. The influence of age on steroidogenic enzyme activities of the rat adrenal gland: enhanced expression of cholesterol side-chain cleavage activity. Endocrinology. 1987;120(6):2521-8.
23. Nomura, Y., Makihata, J. and Segawa, T. (1985). Activation of adenylate cyclase by dopamine, GTP, NaF and forskolin in striatal membranes of neonatal and senescent rats. Eur. J. Pharmacol. 106, 437-440.
24. Sugawa M, May T. Age-related alteration in signal transduction: involvement of the cAMP cascade. Brain Res. 1993;618(1):57-62.