Effect of adrenergic blocking agents on triglyceride content of splenic cells when added alone or with adrenergic catecholamines[a]

| Experiment number | | Drugs | Concentration ($M$) | Content of triglycerides (%) |
|---|---|---|---|---|
| I | 1 | Isoproterenol | $10^{-8}$ | 64.4 |
| | 2 | Propranolol | $10^{-7}$ | 51.1 |
| | 3 | Isoproterenol + Propranolol | $10^{-8}$ + $10^{-7}$ | 21.5 |
| II | 1 | Epinephrine | $10^{-9}$ | 40.4 |
| | 2 | Phentolamine | $10^{-6}$ | 12.4 |
| | 3 | Epinephrine + Phentolamine | $10^{-9}$ + $10^{-6}$ | 11.3 |

[a]Adrenergic catecholamines were added 5 min after the addition of the blockers.

reported by FELLER and FINGER[10] in rat epididymal fat tissue. In marked contrast, under similar conditions none of the adrenergic agents active upon splenic cells caused any significant change in the triglyceride levels of the lymph node cells, even when incubated in concentrations as high as $10^{-5}$ $M$ (triglyceride contents: 39.6 $\pm$ 3.6 mg/g for control cells and 39.0 $\pm$ 3.5 mg/g for drug-treated cells, $p > 0.8$) (Figure). Whether or not this contradiction between the two lymphoid cell populations of different sources merely reflects the vast complexities of each pooled cell population[11–15], remains unclear. The time course of the effects of isoproterenol on splenic cells was followed for ranging up to 6 h. Although the time course considerably varied with concentrations, a measurable decrease in triglyceride contents was produced within 1/2 to 1 h, reaching the maximal response after 2 to 4 h. On 6-hour incubation, the lipid levels rose toward the control levels. In an attempt to determine the mode of action for adrenergic agents, the effects of the catecholamines were evaluated in the presence of $\alpha$- and $\beta$-adrenergic blocking agents. In the Table are given the results of 2 experiments in which isoproterenol, epinephrine, propranolol and phentolamine were added as indicated. Unexpectedly, both propanolol and phentolamine, when added singly in the splenic cell suspensions, caused a dramatic lowering of the lipid levels, and the isoproterenol- or epinephrine-induced reduction of the lipid contents were unaffected by the presence of either of the adrenergic blockers. Interestingly, fatty acids have recently been shown to be involved in corticosteroid-induced lymphocytolysis (TURNELL et al.[16]) and in the regulatory mechanism for cell-mediated immunity by prostaglandins (MERTIN and HUGHES[17]). The present results suggest an involvement of lipid metabolism in physiological functioning of lymphocytes through adrenergic receptor system.


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## Effect of ACTH on the Zona Reticularis of the Rat Adrenal Cortex: an Ultrastructural Stereologic Study


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*Summary.* The effects of ACTH on the rat adrenal *zona reticularis* were investigated by stereologic methods. It was found that the *zona reticularis* cell responsiveness to ACTH is similar to that of the *zona fasciculata* elements. This excludes that the *zona reticularis* of the adult rat can only function as the site of destruction of worn-out elements migrating from the adrenal outer zones.


Although the ultrastructure of the adrenal cortex in normal and experimental conditions was described in numerous investigations[2], until recently very scarce reports appeared concerning the cytophysiology of the adrenal zona reticularis[3]. However, the study of the zona reticularis is of great interest, since at present the role played by this zone in the histophysiology of the entire adrenal gland is still the object of conflicting views, which vary according to the 'zonal hypothesis' or the 'migration theory'[2]. It therefore seemed worth investigating, by stereologic and electron microscopic methods, the response of the rat adrenal zona reticularis to a chronic treatment with ACTH.

*Materials and methods.* 42 young male rats (Wistar-derived) weighing about 200 g, were divided into 7 experimental groups, of which 6 received i.p. injections of 10 IU/kg of ACTH (Sigma) for 3, 6, 9, 12, 24 and 36 consecutive days, respectively. The other group received i.p. injections of normal saline and served as a control. The animals were sacrificed by cervical dislocation.

The right adrenal of each rat was fixed in 10% buffered formalin, embedded in paraffin and serially cut at 7 µm. Sliced pieces of the left gland were fixed in 3% glutaraldehyde, post-fixed in 1% $OsO_4$ and embedded in an epoxy resin. Thick sections were made with LKB III ultramicrotomes and observed with the light microscope for orientation. Thin sections were cut at the level of the zona reticularis and observed in a Hitachi HU-12 or HS-9 electron microscope.

On the serial paraffin sections, the volume of the entire adrenal gland and of the zona reticularis was determined using a method previously detailed[4]. The volume of cells,

Changes in morphometric parameters of rat zona reticularis after ACTH-treatment (means ± SEM)

| Parameters | | Control (6) | 3 days (5) | 6 days (5) | 9 days (6) | 12 days (5) | 24 days (5) | 36 days (6) |
|---|---|---|---|---|---|---|---|---|
| Volume of the zona reticularis | $mm^3$ | 3.58 ± 0.41 | 3.63 ± 0.39 | 4.41 ± 0.42 | 5.41 ± 0.49 | 4.84 ± 0.46 | 5.34 ± 0.43 | 6.28 ± 0.50 |
| | $p$ | | NS | 0.01 | ∨ 0.01 | ∨ 0.01 | ∨ 0.01 | ∨ 0.01 |
| Number of parenchymal cells ($\times 10^6$) | $N$ | 2.61 ± 0.31 | 2.43 ± 0.29 | 2.62 ± 0.29 | 2.84 ± 0.33 | 2.47 ± 0.32 | 2.51 ± 0.28 | 2.64 ± 0.31 |
| | $p$ | | NS | NS | NS | NS | NS | NS |
| Volume of cells | $\mu m^3$ | 1239.8 ± 80.9 | 1332.8 ± 117.7 | 1525.6 ± 161.8 | 1721.1 ± 180.5 | 1814.1 ± 174.2 | 1965.5 ± 163.2 | 2209.1 ± 229.7 |
| | $p$ | | ∨ 0.05 | ∨ 0.01 | ∨ 0.01 | ∨ 0.01 | ∨ 0.01 | ∨ 0.01 |
| Volume of nuclei | $\mu m^3$ | 106.5 ± 9.8 | 127.2 ± 13.4 | 122.3 ± 8.6 | 136.9 ± 10.1 | 140.2 ± 13.2 | 152.1 ± 14.4 | 159.6 ± 16.2 |
| | $p$ | | ∨ 0.01 | ∨ 0.01 | ∨ 0.01 | ∨ 0.01 | ∨ 0.01 | ∨ 0.01 |
| Volume of mitochondrial compartment | $\mu m^3$ | 413.9 ± 30.6 | 450.6 ± 42.9 | 520.0 ± 57.2 | 648.1 ± 70.6 | 724.1 ± 61.3 | 762.1 ± 80.5 | 854.9 ± 86.5 |
| | $p$ | | ∨ 0.05 | ∨ 0.01 | ∨ 0.01 | ∨ 0.01 | ∨ 0.01 | ∨ 0.01 |
| Volume of lipid compartment | $\mu m^3$ | 56.7 ± 6.8 | 77.4 ± 7.5 | 97.4 ± 10.4 | 83.2 ± 10.5 | 100.3 ± 12.6 | 116.5 ± 11.3 | 123.2 ± 13.7 |
| | $p$ | | ∨ 0.01 | ∨ 0.01 | ∨ 0.01 | ∨ 0.01 | ∨ 0.01 | ∨ 0.01 |
| Surface of mitochondrial cristae | $\mu m^2$ | 2069.5 ± 187.3 | 2207.9 ± 210.8 | 2544.9 ± 249.1 | 3110.8 ± 310.4 | 3664.3 ± 317.4 | 3833.2 ± 361.5 | 4274.5 ± 400.6 |
| | $p$ | | NS | 0.01 | ∨ 0.01 | ∨ 0.01 | ∨ 0.01 | ∨ 0.01 |
| Surface of SER | $\mu m^2$ | 6857.5 ± 612.8 | 7422.8 ± 701.0 | 8852.7 ± 795.7 | 9380.8 ± 972.2 | 9837.9 ± 1001.6 | 10671.8 ± 1037.4 | 12092.3 ± 1307.5 |
| | $p$ | | ∨ 0.05 | ∨ 0.01 | ∨ 0.01 | ∨ 0.01 | ∨ 0.01 | ∨ 0.01 |

Animals were treated as described in the text. The number of animals in each group is indicated in parentheses. $p$, level of significance of the difference between the control and ACTH-treated groups; NS, not significant ($p > 0.05$).

nuclei and of the various subcellular compartments, as well as the surface of smooth endoplasmic reticulum (SER) and mitochondrial cristae, and the % of the volume of the zona reticularis occupied by sinusoids, were estimated from light and electron micrographs using the morphometric techniques and sampling procedures previously described[5,6]. The number of cells contained in the zona reticularis was determined by dividing the volume of the zona reticularis occupied by parenchymal cells for the mean cell volume.

Student's $t$-test was used for the statistical evaluation of results. All the statistical procedures were performed with an electronic microcomputer (Olivetti No. P652/ROM 01-MLU600).

*Results and discussion.* The structure of intact rat zona reticularis was described in detail recently[3] and therefore it will be reviewed here only briefly since our data almost completely agree with the previous ones.

The zona reticularis of the control rats consisted of short cords of irregularly shaped cells intermingled with very large sinusoids, and had a width ranging between 9–15 µm. The cells of the zona reticularis did not show ultrastructural differences in respect to those of the zona fasciculata, with the exception of their smaller volume and paucity in lipid droplets. Mitochondria were round or ovoid and contained tubular or tubulo-vesicular cristae; SER profiles were quite abundant and the Golgi apparatus well developed. The electron dense bodies of lisosomal nature were very scarse. Degenerated or death cells, described previously[7], were only found exceptionally.

After ACTH treatment the volume of the zona reticularis and the volume of parenchymal cells were found to be significantly increased, whereas the number of cells of the zona reticularis did not vary (Table). This indicates that the ACTH-induced volumetric increase of the zona reticularis is due only to the cell hypertrophy and excludes that the ACTH-elicited zona fasciculata cell proliferation[6] might significantly contribute to the zona reticularis growth.

In the electron micrographs, no conspicuous qualitative changes were noted after ACTH-administration: the cells showed only a hypertrophied Golgi apparatus. Quantitative morphometric data (Table) indicated that the volume of nuclei, and of mitochondrial and lipid compartments as well as the surface of SER and mitochondrial cristae were significantly increased in relation to the duration of ACTH-treatment. It must be stressed that these changes are completely analogous to those elicited by the hormone in the cells of the zona fasciculata[6], and, according to our previous contributions[6,8,9], they can be interpreted to represent the morphological counterpart of the ACTH-induced enhancement of the growth and steroidogenic capacity of the rat adrenal zona reticularis cells.

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To summarize, the present investigations has shown that in the zona reticularis of the young adult rat a) the images suggesting cell degeneration are extremely rare; b) the cells display all the morphological features of the actively steroidsecreting elements; c) the cell responsiveness to ACTH is quite similar to that of the zona fasciculata elements.

In conclusion, these results and the demonstration that the cells of the zona reticularis are well endowed with ACTH-receptors[10], support the view that the zona reticularis, at least in the adult rats, cannot be merely considered the site of destruction of worn-out elements migrating from the outer zones, but that this zone is actively involved in steroid synthesis. The differences in the morphology and proliferative capacity between the cells of the zona fasciculata and zona reticularis require, however, further investigations to settle their functional significance.

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## Struktur-Wirkungsbeziehungen beim menschlichen Calcitonin. III.[1] Die biologische Aktivität verkürzter oder an den Kettenenden modifizierter, synthetischer Analoger[2]

## Structure-Activity Relationship of Human Calcitonin. III. Biological Activity of Synthetic Analogues with Shortened or Terminally Modified Peptide Chains

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*Summary.* Assays of 8 synthetic analogues of human calcitonin in rats showed that their hypocalcaemic activity was drastically reduced by deletion of the C-terminal amide group, chain-shortening or opening of the disulphide ring, but unaffected or enhanced by modification of the N-terminal amino group.

Die bisher aus verschiedenen Arten isolierten Calcitonine[3] weisen überraschend grosse Unterschiede in ihren Aminosäuresequenzen auf. Dagegen besitzen alle als gemeinsame Strukturmerkmale eine Kette von 32 Aminosäuren mit einem C-terminalen Prolin-amidrest, sowie einen Disulfidring zwischen den Halbcystinresten in den Positionen 1 und 7. Die Tatsache, dass Amino- und Carboxylene unverändert erhalten werden, trotz einer hohen Austauschrate der übrigen Aminosäuren, deutet darauf hin, dass die Kettenenden zur Erhaltung der biologischen Aktivität notwendig sind. Mit dieser Annahme stimmen unsere früher[4] bei Abwandlung von Schweinecalcitonin (PCT)-peptiden gemachten Beobachtungen überein. Wir haben zeigen können, dass bereits geringfügige Änderungen am Carboxylende von PCT die biologische Aktivität stark erniedrigen.

Eine entsprechende Untersuchung wurde jetzt auch auf menschliches Calcitonin (HCT, Calcitonin M[5]) ausgedehnt. Dazu dienten die in der Tabelle angegebenen synthetischen Analogen, die entweder eine verkürzte Peptidkette oder Abwandlung an den Kettenenden aufweisen.

Diese Substanzreihe umfasst als C-terminal veränderte Peptide die freie C-terminale Säure HCT-(1-32)-OH, den entsprechenden Methylester HCT-(1-32)-OMe und die um eine beziehungsweise drei Aminosäuren verkürzten Sequenzen HCT-(1-31)-$NH_2$ und [$Pro^{29}$]-HCT-(1-29)-$NH_2$. In letzterem Analogen ist der natürlicherweise in Stellung 29 vorkommende Valin- durch einen Prolinrest ersetzt. Dadurch sollte abgeklärt werden, ob Aktivität erhalten bleibt, falls bei verkürzter Kette eine C-terminale Prolinamidfunktion vorhanden ist, wie beim HCT selbst. [$Ser^2$, Des-$Gly^{10}$]-HCT ist um eine Aminosäure ($Gly^{10}$) im Ketteninnern verkürzt, enthält also die Calcitonin-Sequenzen 1–9 und 11–32 miteinander verknüpft. Zudem enthält dieses Analoge noch einen Serin-Rest in Stellung 2, wie er bei den andern Säuger-Calcitoninen auftritt. Als N-terminal abgewandelte Sequenzen wurden [$Bmp^1$]-HCT, [$N^{\alpha}$-Acetyl-$Cys^1$]-HCT sowie die offenkettige Sequenz [$NMet^{1,7}$]-HCT in der die beiden Cysteinreste in Stellung 1 und 7 methyliert sind, dargestellt.

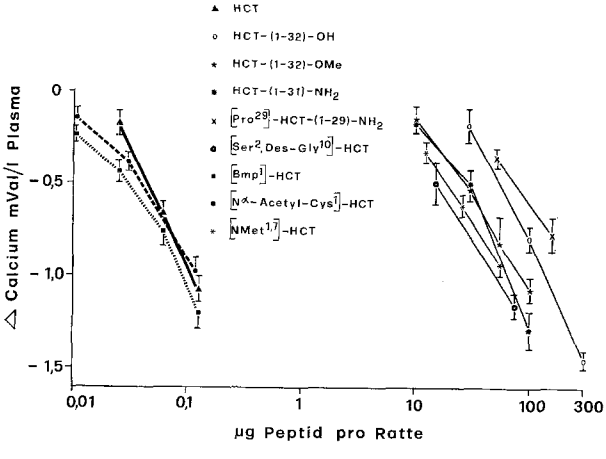


Fig. 1. Dosis-Wirkungsbeziehung der hypocalcaemischen Aktivität gemessen 50 Min nach i.v. Applikation der Peptide. Jeder Punkt repräsentiert das Mittel aus 15 Messwerten (± Standardfehler).

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