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Changes of the adrenal medulla of rats were studied in the course of adaptation to repeated immobilization stress. An increase in the number of cells in the adrenal medulla was found in the adapted animals; this increase was confirmed by weight indices of the medulla and by cell counts per surface unit.

Simultaneous karyometric measurements of the nuclei of adrenal medulla cells and an analysis of the catecholamine contents in the adrenals attest to the increased activity of the adrenal medulla in the course of adaptation.
CATECHOLAMINES OF THE ADRENAL MEDULLA AND ITS MORPHOLOGICAL CHANGES DURING ADAPTATION TO REPEATED IMMOBILIZATION STRESS

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The role of catecholamines in acute stress situations, as well as in adaptation to repeated stress, is nowadays generally considered to be of importance. The contents of catecholamines (further KCHA) in the adrenal medulla (further NO), as well as their excretion through urine during acute stress and in adaptation to prolonged stress have heretofore been studied primarily on models of exposure to cold stress[1, 2, 5, 8, 9, 11]. According to Euler, a single exposure to cold stress produces fluxion of KCHA into blood, whereby there occurs a depletion of KCHA in the adrenal NO. According to the same author, in animals adapted to cold the contents of KCHA in the adrenal NO increases to its normal level or sometimes even exceeds it.

It has been documented that the weight of NO in animals exposed to repeated stress significantly increases [14]. We also noted in our previous experiments that when rats were immobilized daily for 2.5 hours, the weight of their medulla increased in just 10-14 days by as much as 100 percent [10].

However, when we want to study the functional manifestations of the adrenal NO, specifically its contents of KCHA, we are faced with answering the basic question whether the above mentioned enlargement of the medulla also applies to its adrenal part.

Morin and Schaeffer established on the basis of a cold stress model that the adrenal NO in rats adapted to cold undergoes hypertrophy
in parallel with the cortex of NO. However, this finding was not confirmed by Heroux, who ascribed increases in the weight of NO in chronic cold stress solely to hypertrophy of the cortical zone fasciculata, without any changes in the adrenal part.

As literary data relevant to this problem tend to be contradictory and, as regards their number, scarce, we decided to study this problem using our own experimental materiel. We studied the contents of catecholamines in the adrenal medulla as well as morphological changes in the medulla in the course of adaptation to repeated immobilization stress.

**Materiel and Methods**

We used rats of the Wistar strain, males weighing 180-200 g from the breeding station at Lysolaje; the rats were fed Larsen’s diet and received tap water ad libitum.

As experimental model we used immobilization stress, whereby we fixated the animals by adhesive bands to a solid plate daily for 2.5 hours (further referred to only as “fixation”).

The animals were divided into three groups: a) control group (6 rats); b) group exposed to three days of fixation (7 rats), that period having been selected on the basis of previous findings, as after that period occurs maximum depletion of KCHA 7; c) group exposed to 45 days of fixation, where we considered the rats to have become adapted (9 animals). In each animal we determined KCHA in the right NO and the left NO we used for isolation of medulla, which we then subjected to histological analysis.

The contents of KCHA in NO was established fluorometrically following their isolation on aluminum oxide. Our methodology was based
on a suitable combination of methods devised by Euler and Lishajko [3, 4], Smetana and Dlohoska. As a standard we used the substance adrenalin (Lachema product) for expressing the entire contents of KCHA.

In order for us to weigh the isolated medulla, we devised a method for decortication of NO. After decapitating the animal we quickly removed NO, weighed it and immediately froze it by solid CO2. Then, under a preparation loupe, we gradually separated the darker hull till we exposed the compact lighter medulla. The decortication was always done by the same person and under identical conditions so that any eventual innacuracy in preparation would be the same in all groups. The degree of decortication was verified by histological analysis, whereby we fixated the isolated adrenal medulla in neutral formalin and then embedded it in paraffin. Cuts were stained by hematoxylin and eosin. In determination of the cell count we used an ocular screen with a 5 mm edge. We counted all cells in six squares, i.e., in an area of 150 mm² for each experimental animal. The size of the cell nuclei of adrenal medulla was measured under overall 3,000 X magnification. The volumes of the nuclei were computed according to tables listed by Palkovits.

The findings were subjected to t tests according to Student.

Results

As can be seen in figure 1, the weight (of one) adrenal in the course of adaptation to repeated 45-day fixation increased significantly (P less than 0.001) as compared to the controls and the animals fixated for 3 days (P less than 0.001). We knew that this was due to growth of the cortical part of NO, but we did not know what
would be the behavior of the NO; would it remain the same, or would it increase in parallel with the cortex.

Figure #1. Average weights of medullae and whole adrenals during adaptation to repeated fixation plus or minus sigma (SD). K = control.

Key: (1) adrenal in mg
(2) medulla NO in mg
(3) percent of medulla in NO

The central part of figure 1 shows that the weight of adrenal medulla in the case of rats fixated for 45 days significantly, with $P$ less than 0.01, differs from that of the controls as well as from that of animals fixated for three days ($P$ less than 0.001). Weight of medullae in rats fixated for three days shows practically no difference in comparison to the control animals. Expressing the weight of the medulla in relation to the overall weight of the adrenal in percentages yields a practically identical result for all of the three experimental groups which, as can be seen in the bottom part of figure 1, is around six percent. Thus, percentage of weight of the medulla in relation to that of the adrenal shows no differences neither in the case of animals fixated for three days, nor for the animals fixated for 45 days. Thus, we can conclude that increase in
the weight of the adrenal is accompanied in parallel by that of the
cortex as well as the medulla. The degree of decortication and the
time of the cell nuclei of the adrenal medulla of control animals
and of animals fixated for 45 days is reproduced in photographs la,
b, c. The increased weight of adrenal medulla, however, tells us
nothing about the distribution of cells per area unit. Thus we were
interested in finding out whether there occurs any increase or en-
largement. Our findings showed that the number of cells in the ad-
renal medulla per area unit does not show any significant difference
during fixation. Thus, we further conducted karyometric measurement
of the size of cell nuclei of the adrenal medulla in control animals
and in animals fixated for 45 days. We determined that the volume
of cell nuclei increases significantly in animals fixated for 45
days in comparison to the values obtained for the control animals
(P less than 0.02), which indicates an obvious hypertrophy of cells
in the adrenal medulla. Overall, it can then be stated that this
hypertrophy of adrenal medulla cells occurs in animals that have be-
come adapted and on that basis we can then explain also weight in-
creases in the cortex of the adrenal.

In connection with studying the problem of hormone biochemistry
in the adrenal medulla during repeated stress, we determined the o-
verall contents of KCHA in adrenal preparations.

Absolute values of KCHA in NO are shown in the upper portion of
figure 2. Their level increases very significantly (P less than 0.01)
in the case of rats fixated for 45 days in comparison to the control
animals or animals fixated for three days (P less than 0.001). After
the third day of fixation the level of KCHA decreases, but not enough
Photograph 1. Overall View of Decorticated Adrenal

la: 24 X magnification

D - medulla
K - cortex

Large Magnification of Cells of Adrenal Medulla in

lb: Control Animals

lc: In animals fixated for 45 days

320 X magnification
Figure #2. Average levels of catecholamines during adaptation to repeated fixation plus or minus sigma (SD). K - control group. (1) micrograms per 1 kilogram of body weight.

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to be statistically significant. The central part of figure 2 shows the concentration of KCHA in 100 mg of NO tissue. It indicates a decrease in the case of animals fixated for 3 days and a return to essentially control group levels in animals fixated for 45 days. However, the results are not statistically significant. The bottom part of figure 2 shows the contents of KCHA in one NO per 1 kg of body weight. In adapted animals the level of adrenal KCHA (P less than 0.001) per 1 kg of body weight was approximately twice that of control animals or that of animals that have not yet become adapted. (Body weights in g: control 207 plus-minus 16; fixated for 3 days 186 plus-minus 14; fixated for 45 days 162 plus-minus 14.)

Discussion

As already stated, Morin and Schaeffer described hypertrophy of NO cortex during prolonged exposure to cold. Their findings were not confirmed by Heroux, who also used the cold exposure model and stated
that the increase in the weight of NO is due solely to cortex growth. He even took a count of cells per area unit, but found no difference between the medullae of adapted and nonadapted rats.

We also did not succeed in documenting that an increase occurs in the number of cells per area unit in adapted animals. The finding of increased weight of NO cortex we interpret as an absolute multiplication of cells in the entire medulla. The significant increase in the volume of cell nuclei of the NO cortex, which we arrived at through karyometric measurements of adapted animals, forms a morphological basis for explaining the increased level of KCHA in NO, i.e., also increased activity of adrenal medulla cells.

As regards the level of catecholamines in adrenal medulla, our findings agree with literary data, even though we again make comparisons with cold exposure models [2, 11]. Desmarais and Dugal exposed rats to 0°C for up to 75 days and determined adrenalin and noradrenalin in NO separately. They established that following initial depletion of both KCHA in NO medulla (1-2 days), the adrenalin (ADR) level on the 24th day of exposure to cold returned to normal and ceased to change, while the level of noradrenalin (NA) in the medulla kept gradually increasing up to the 75th day, but already on the 24th day it was far above normal. The importance of NA in adaptation to cold was confirmed also by Leblanc and Pouliot who, after repeated administration of NA to animals at room temperature, noted that their oxygen consumption was analogous to that of animals adapted to cold (45 days). The significance of NA in adaptation to cold is also stressed by Leduc.
Our findings confirm that the level of KCHA in the adrenal medulla of adapted animals is much higher than it is in controls. After computation per unit of NO tissue the KCHA level does not differ from that found in control animals, but if we take into consideration the fact that the medulla in adapted animals is significantly larger, we can see that the adapted organism has much more of them at its disposal. An identical conclusion is arrived at through computation of the level of KCHA in NO per unit of body weight.

In comparing the indices of cortex activity of adrenals from previous experiments with KCHA levels in NO determined in this experiment we find that they do not progress in parallel. While the contents of corticosterone in NO increases already during the first few days of repeated fixation, the KCHA level in NO shows an almost contrary progression. This statement is important from the viewpoint of the still unexplained role of KCHA in activation of adrenocortical reaction to stress. Another, equally interesting aspect of the problem of the role of KCHA in adaptation is given by the varying physiological and pharmacodynamic effects of adrenalin and noradrenalin. Adrenalin affects primarily metabolism, while noradrenalin effects are more hemodynamic. For gaining a better understanding of the role of KCHA in adaptation of organisms it will be necessary to study the behavior of adrenalin and noradrenalin separately.

Conclusion

We used the model of repeated immobilization stress for studying the behavior of the adrenal medulla in rats in the process of adaptation.
We determined an increase in the number of cells in the adrenal medulla of adapted animals which was confirmed by weight indices of the medulla and by cell count per area unit.

Simultaneous karyometric measurements of cell nuclei of the adrenal medulla, as well as analysis of the contents of catecholamines in the adrenal confirmed the increased activity of the adrenal medulla in the course of adaptation.
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