ULTRASTRUCTURE OF SYNAPSES IN THE SUPRAOPTIC NUCLEUS OF THE CAT AFTER INDUCED HYPOTHERMIA

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Ultrastructure of axosomatic (AS) and axodendritic (AD) synapses in the supraoptic nucleus (NSO) was investigated in a group of normothermic cats and compared with another group of cats after short-term induced hypothermia. Quantitative analysis demonstrated a significant decrease of number of AS synapses, smaller size of AD synaptic knobs, shorter length of the synaptic contact and an increase of the active zone. Evaluation of the shape of the synaptic cleft demonstrated an increase of both positive (P) and negative (N) types in hypothermic animals. The observed morphological changes can be ascribed to the decreased synaptic activity in a greater part of the synaptic population and to the increased activity in a smaller portion of synapses.

INTRODUCTION

The supraoptic nucleus (NSO) represents a very important integrative region of the hypothalamus and it has a great significance in the system of the neurohumoral regulations. Its activity is influenced by many afferent impulses comming from different CNS areas [1, 2, 3]. Beside the ascendent and descendent nerve fibres the NSO is influenced by humoral factors realized via small interneurons, such as the osmoreceptors of Verney and the LCN (liquor contacting neurons). That is why the NSO was subjected to many morphological and experimental studies. One of them was our previous paper[4], in which we described the changes of synapses in the NSO of the hedgehog after long-term natural hibernation. Obtained morphological results demonstrated an increased synaptic activity in the NSO after hibernation, but in the brain cortex[5] the changes indicated a decreased synaptic activity.

Therefore we decided in the present work to study the changes of synaptic apparatus in the cat, as a non-hibernating animal after short-term induced hypothermia.

MATERIAL AND METHODS

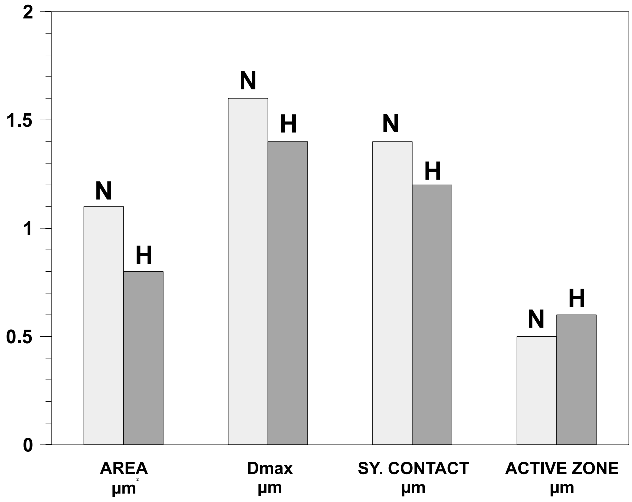
Brains of six adult cats (weight 2,800–3,500 g) were used for this study. The first three animals served as a control and they were investigated under normal body temperature (mean rectal temperature 38.5 °C). Next three were studied after deep hypothermia, induced by application of a lytic mixture of chlorpromazine (50mg/kg) with dihydroergotonin (10mg/kg). The rectal temperature was thus lowered to 19.2–20.7 °C for the time interval of 5 hours.

In all experimental animals the material was handled by the same methods. In the pentobarbital anesthesia the brains were fixed in situ by perfusion of the vascular bed by a mixture of 2% glutaraldehyde with 1% formaldehyde in a phosphate buffer of pH 7.3. The skul was then fixed in a stereotactic apparatus and after removal of the superficial cranial bones two frontal sections were made corresponding to the stereotactic levels A10 and A20. This area corresponds to the location of optic chiasma and adhering areas. The removed tissue block of hypothalamus was divided by means of a tissue chopper into slices 0.5 μm thick. Under the dissecting microscope the areas corresponding to the location of the NSO were cut out. These blocks were additionally fixed in the same fixation mixture of aldehydes, followed by fixation in 2% osmic acid, dehydrated in acetone and embedded in Durcupan ACM (Fluka). Semithin section 0.5mm thick were stained by toluidine blue and investigated under the light microscope. From selected areas ultrathin sections were cut and investigated under the electron microscopes TESLA BS613 or Zeis Opton 109.

For the quantitative analysis of the axosomatic (AS) synapses pictures were taken at direct magnification of 4,700 × and additionally enlarged to the total magnification of 21,000 ×. The outlines of the perikarya and of the AS synapses on their surfaces were drawn on a sheat of paper and measured by means of the picture analyser

Videoplan (Kontron). We measured the total length of the superficial membrane of the perikarya, the areas of the synaptic knobs, the length of the synaptic contacts and the length of the active zones in individual synapses. Then we calculated the number of synapses per unit length of 1 mm of the surface of the perikaryon covered by synapses.

The quantitative evaluation of axodentritic (AD) synapses was performed on pictures at total magnification of 50,000 × by the same picture analyser. We measured the area, perimeter, maximal diameter and form factor of the synaptic knobs and the length of the synaptic contact and of the active zone. Moreover we evaluated the curvature of the synaptic cleft according to Jones[6] and calculated the percentual proportions between the positive (P), flat (F) and negative (N) types of the synapses.



Graph 2. Arithmetical mean of the area, maximal diameter, length of the synaptic contact and lenght of the active zone in the AD – synapses. N – normothermic, H – hypothermic cats.

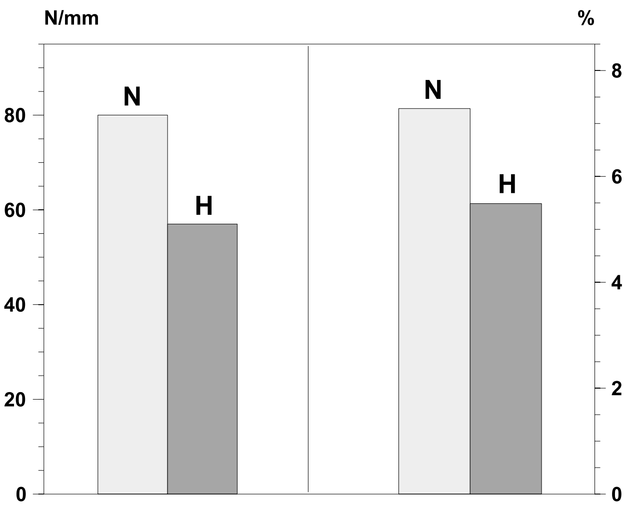
RESULTS

The axosomatic synapses

Neurosecretory neurons in the NSO are rather large cells, but they contain relatively small number of the AS synapses on their surface. The synaptic knobs are of the typical spherical shape and they contain rather large number of light spherical synaptic vesicles and small number of dense cored vesicles.

The quantitative evaluation of the number of synapses in normal cats and in the cats after hypothermia is demonstrated in the Graph 1. From this graph it is evident, that the number of AS synapses in the normal cat is rather low – only about 80 synapses per unit length of 1 mm, which corresponds to 7% of the surface of the perikaryon.

In the cats after deep short-term hypothermia the number of AS synapses is significantly lower, as it is shown in the Graph 1.

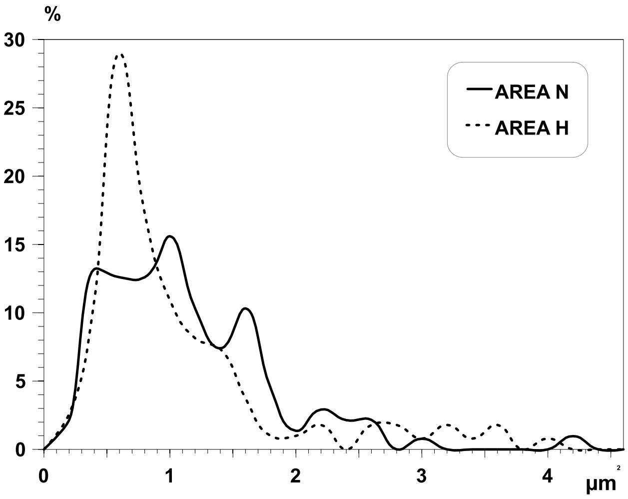


Graph 1. Axosomatic synapses. Left – number of synaptic knobs per unit length of 1 mm of the surface of the perikaryon. Right – percentual proportion of the perikaryon surface covered by synapses. N – normothermic, H – hypothermic cats.

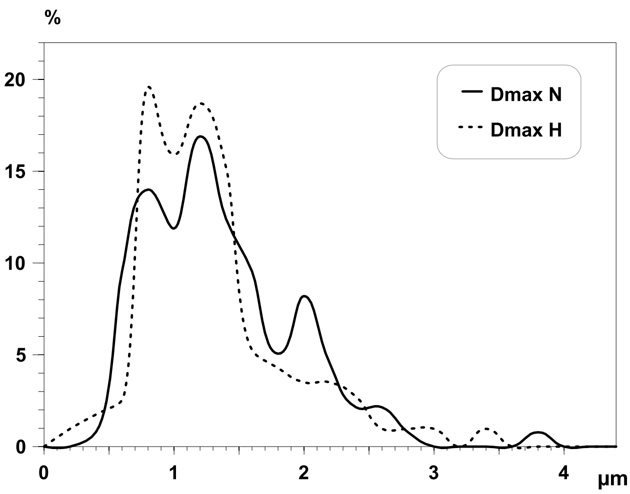
The axodendritic synapses

The neuropil of the NSO is formed by a dense network of nerve fibres, mostly thick unmyelinated dendrites, covered by numerous AD synapses, myelinated and unmyelinated axons,and a smaller number of glial processes. The results of the quantitative analysis of the AD synapses are summarized in Graph 2, which demonstrates the mean values of the area and maximal diameter of the synaptic knobs and the length of the synaptic contact and of the active zone.

The presented data show, that in cats after hypothermia the size of the synaptic knobs is smaller, as it is evident from lower values of the area and maximal diameter. The diferences of the size of synaptic knobs are more evident by comparison of the curves, showing the distribution of the area (Graph 3) and maximal diameter (Graph 4). In hypothermic animals both curves are shifted to the left, that means to the lower values.



Graph 3. Curves showing the distribution of area of the AD – synapses. N – normothermic, H – hypothermic cats.

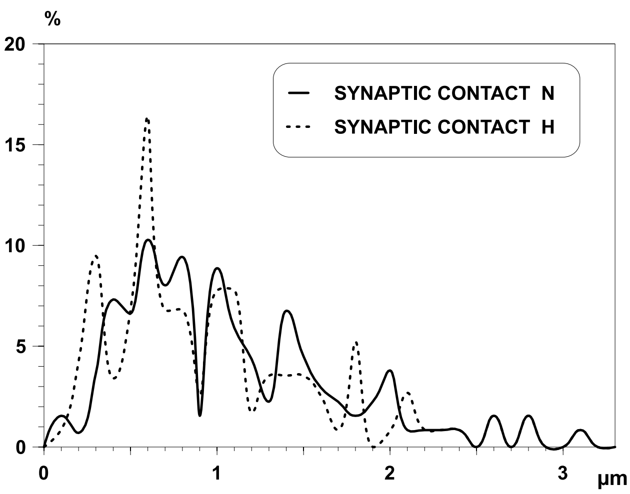


Graph 4. Curves showing the distribution of maximal diameter of the AD synapses. N – normothermic, H – hypothermic cats.

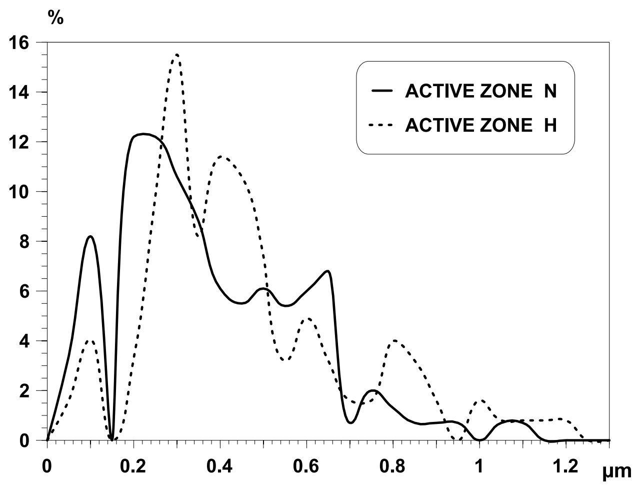
The values of the form factor did not show any significant differences between the normothermic and hypothermic cats, which indicates that the shape of the synaptic knobs does not change.

The length of the synaptic contact was also smaller in the cats after hypothermia, as it is shown by lower mean values, demonstrated in the Graph 2 and by the different shape of the distribution curves in the normal cats and after hypothermia, shown in the Graph 5. From the last mentioned graph it is also evident, that the values of the length of the synaptic contact show rather great variability.

The mean values of the length of the active zone were higher in the cats after hypothermia (Graph 2). The curves, showing the distribution of the length of the active zone demonstrated in the Graph 6, show great variability of these values, which results in the fact, (Graph 6) that in some synapses the lengt of the active zone after hypothermia is increased, but in other decreased.

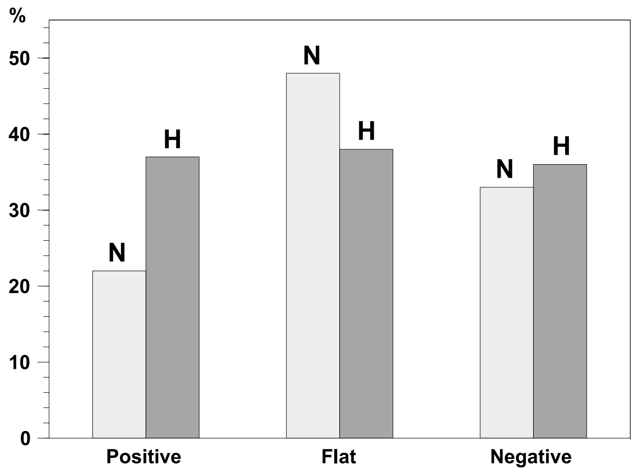


Graph 5. Curves showing the distribution of the lenght of the synaptic contacts of the AD synapses. N – normothermic, H – hypothermic cats.



Graph 6. Curves showing the distribution of the lenght of the active zone of the AD synapses. N – normothermic, H – hypothermic cats.

Next quantitative analysis concerned the classification of AD synapses according to the curvature of the synaptic cleft according to Jones[6]. The results, presented in the Graph 7, show that in the normal cats the greates numer corresponds to the synapses of the F-type, lower number to the P-type synapses and less numerous are the synapses of the N-type. After the hypothermia these proportions were changed. We noticed an increase of the number of synapses of the P-type and the N-type, with relative decrease of the F-type synapses.



Graph 7. Percentual proportions between the positive, flat and negative types of the AD synapses in N – normothermic and H – hypothermic cats.

DISCUSSION

The present quantitative analysis of the synapses in the NSO in normal cat demonstrated marked differences in the number of AS and AD synapses. The AS synapses were less numerous than the AD and their number was even decreased after hypothermia. From the literature[1, 2, 3] it is known, that the NSO receives great number of afferent impulses. So it is evident, that only a small number of afferents terminates directly on the surface of the perikarya and the greatest portion terminates on the dendrites.

The AD synapses show after the hypothermia a decrease of their size, accompanied by shortening of the length of the synaptic contact, which can be explained by a lowered synaptic activity. In contradiction to this is the greater length of the active zone. The distribution curves show a great variability of the mentioned values of the active zone. We suppose, that this finding can be explained by the presence of functionally different types of the AD synapses in the neuropil of the NSO.

In accordance with this statement are the results obtained at evaluation of the curvature of the synaptic cleft. After hypothermia we observed significant increases of the positive (P-type) synapses, which are taken[6] as the synapses with lower activity as well as an increase of the negative (N-type) synapses, functionally explained as synapses with higher activity.

In our previous paper[4] we studied the changes of the synaptic apparatus in NSO of the hedgehog after the long-term natural hibernation. We demonstrated significant increase of the length of the active zone and greater number of the negative type of synapses. Described changes gave evidence of the increased synaptic activity in the NSO during hibernation. Present results in the cat after induced hypothermia showed on the contrary a decreased synaptic activity in the NSO. From that it is evident, that the induced short-term hypothermia induces different changes of the synaptic apparatus and is not equivalent to the long-term natural hibernation.

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