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Pregledni članak

EFFECTS OF STRESS ON NEUROENDOCRINE RESPONSES

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ABSTRACT

Maintenance of adequate levels of response of the hypothalamic-pituitary-adrenal axis during stress is important for survival. The level of response of the pituitary corticotroph is determined by differental regulation of the hypothalamic regulators, corticotropin-releasing hormone (CRF) and vasopresin (VP), and the senzitivity of the negative glucocorticoid feedback. Although the exact interaction between regulatory factors and the molecular mechanisms controlling the sensitivity of the corticotroph during stress remain to be determined, it is clear that regulation of the proportional secretion of CRF and VP in the paraventricular nucleus (PVN) of the hypothalamus, modulation of pituitary VP receptors, and the sensitivity to feedback inhibition play a critical role.

Stresful stimuli inhibit testicular function including significant decrease of circulating testosterone. In particular there is evidence that the response of the pituitary-testicular system to aversive stimuli is potentially biphasic, with an initial stimulatory phase and, if stress is prolonged or of sufficient magnitude, a subsequent inhibitory phase. The mechanisms mediating the stress response of the hypothalamic-pituitary-testicular axis are mediated at the level of GnRH-secreting neurons, while long-term effects also involve preipheral mechanisms such as alteration in pituitary and gonadal responsiveness. Effect of the acute stress are mainly pronounced at the testicular level (steroidogenic enzymes), since decrease of testosterone may occur in stressed animals in the presence of normal circulating LH.

Key words: Adrenocorticotropic hormone; Hypothalamic-pituitary-adrenal axis; Vasopresin; Corticotropin-releasing hormone; Hypothalamic-pituitary-testicular axis; Gonadotropin-releasing hormone; steroidogenesis; stress.

INTRODUCTION

The constancy of the internal milieu of homeostasis is critical for survival and independent existence of higher organisms. The preservation of this internal environment requires continuous adaptation to external or internal stimuli or stressors, involving behavioral, visceral, and endocrine changes necessary for controlling the disturbance. The major endocrine mechanism is the activation of the hypothalamic-pituitary-adrenal axis (HPA axis), resulting in rapid

increases in circulating ACTH with the subsequent rise in glucocorticoids, which is critical for successful adaptation (Dallman *et al*, 1992; Munck *et al*, 1984.)

The early observation by Selye (1939) that stress is accompanied by both an increase in the activity of the HPA and decrease in reproductive functions (a phenomenon he attributed to the necessity, in case of emergency, of preserving adrenal cortex at the expense of gonadal activity) had suggested a possible relationship between hormones of the HPA axis and those of the hypothalamic-pituitary-gonadal (HPG) axis. Indeed, corticotrophin-releasing factor (CRF), pro-opiomelanocortin (POMC)-derived peptides (such as ACTH and β-endorphin), and adrenal corticosteroides play a very important role in modulating the effect of stress on reproductive functions.

Clearly there are limitations in extrapolating data across species, and differences between species have been identified. For example, in the rat μ-opiate agonists stimulate the HPA axis whereas in humans these are considered inhibitory. However, for a variety of ethical and practical reasons most of the work underlying our understanding of stress mechanisms come from animal studies. This is particularly true of studies at the central level. Consequently we have chosen to concentrate this review predominantly on studies of the rat.

This review will first describe the patterns of ACTH response during adpatation to different types of acute and chronic stress and then discuss hypothalamic and pituitary mechanisms involved in the regulation of ACTH secretion during adaptation to chronic stress. Also this article reviews the mechanisms believed to mediate stress-induced inhibition of reproductive functions.

Stress and the hypothalamic-pituitary-adrenal axis

Acute stress

A wide variety of acute stressors have been used as stimuli for HPA axis activity. These include cold, ether, footshock, s.c. formalin, i.p. histamine, i.p. hypertonic saline, insulin-induced hypoglycemia, restraint, swimming and laparotomy or surgical stress. The common endpoint of these challenges is an increase in circulating ACTH from anterior pititary and increased secretion of corticosterone from the adrenal gland. The HPA axis is very sensitive to mild stress and responds in a graded manner to greater intensities of stress, resulting in increased circulating levels of ACTH and corticosterone. Thus, increasing the volume of experimentally induced arterial hemorrhage results in a related increase in plasma ACTH (Gan *et al*, 1978). Similarly, increasing the challenge of series of low-level psychological stressors (Armario *et al*, 1986) or increasing the intensity of current delivered in footshock (Kant *et al*, 1983) results in an incremental increase in plasma corticosterone. However, while circulating hormones may provide an index of mild stress, the range is very limited and reaches an effective maximum relatively rapidly. This prevents the use of circulating levels of hormone to differentiate between different stressors such as cold, s.c. formalin, restraint etc. (Kant *et al*, 1982).

Mechanisms of CRF Expression and Release during Stress

At the hypothalamic level, acute stress result in a rapid transient increase in bioassayable or immunoreactive CRF in the median eminence, lasting 2-4 min (Buckingam, 1979; Murakami *et al*, 1989). This presumably reflects the activation of mechanisms increasing the transport and/or mobilization of CRF, as the time-course involved is too rapid to reflect increased synthesis. At 15-30 min after the onset of acute stress, median eminence CRF levels decline, presumably reflecting an increase in release of CRF-41 into the HPB (hypophiseal portal blood) (Moldow *et al*, 1987; Suda *et al*, 1988). The increase in CRF release has been confirmed in the push-pull cannulated median eminence of conscious free-moving rats (Ixart *et al*, 1987). At 60-80 min after onset of stress there is a second peak in release (Moldow *et al*, 1987). This second peak probably reflects increased synthesis, as the rise could be prevented by

pretreatment with a protein synthesis inhibitor (Moldow *et al*, 1987). Hypothalamic CRF, determined immunocytochemically, has also been reported to increase 6h after hypertonic-saline stress, which correlates well with the increase in CRF mRNA in the pPVN (parvocellular cells of the paraventricular nucleus) (Lightman & Young, 1988). This same stress has been shown to increase AVP (arginine vasopresin) mRNA in the pPVN without affecting magnocellular AVP mRNA (Lightman & Young, 1988). However, the changes in immunoreactivity may depend on the nature of the stressor as, following restraint stress, Haas & George (1988) did not report an increase in CRF until 24h later.

Increased CRF mRNA has been reported after footschock, insulin-induced hypoglycemia, naloxone-induced morphine withdrawal, restraint, swimming and urethane anesthesia (Lightman & Young, 1988; Suda *et al.* 1988; Harbuz & Lightman, 1989; Harbuz *et al.* 1991). POMC mRNA in the anterior pituitary has been shown to be increased in response to a wide variety of acute stressors, e.g. cold (Wu & Childs, 1991), footshock (Shiomi *et al.*, 1986) hypertonic saline (Ligthman & Young, 1988; Harbuz & Lightman, 1989), insulin-induced hypoglycemia (Tozawa *et al.*, 1988) and restraint (Harbuz & Lightman, 1989).

The mechanisms controlling the expression and release of these regulatory peptides in PVN are complex, with a number of neurotransmitters including, catecholamines, GABA, serotonin, acetylcholine, and several neuropeptides being implicated (Al-Damluji S., 1993; Antoni, 1990; Guillaume, 1987). Of these factors, catecholamines appear to play a major role (Al-Damluji S., 1993; Guillaume, 1987; Plotsky, 1989). CRF neurons receive dense adrenergic and noradrenergic innervation from the brain stem, and adrenergic receptors including α1-, α2-, β1-, and β2- have been demonstrated in the PVN (Cummings & Seybold 1988).

In summary, acute stress result in an immediate (1-4 min) increase in median eminence CRF which is released from neurosecretory vesicles into the HPB. The release of CRF is associated with an increase in synthesis demonstrated by an increase in CRF mRNA. The peptide is carried in the HPB to the anterior pituitary where is acts in concert with other secretagogues on corticotrophs to evoke the release of ACTH and induce POMC gene expression. CRF may act in either a passive or dynamic fashion, depending on the nature of the stressor. This probably reflects the composition of the secretagogue cocktail present in the HPB. Following release of ACTH, there is a subsequent increase in circulating corticosterone levels.

Chronic stress

The study of chronic stress poses two related problems. First is the lack of a suitable model. Most studies concerned with chronic stress utilize an acute stress repeated for a number of days. The second is that with repeated stress there is an habituation or adaptation of the HPA axis resulting in attenuated responses, e.g. with repeated footshock (Kant *et al.*, 1985), restraint (Hashimoto *et al.*, 1988) or ethanol stress (Spencer & McEwen, 1990) plasma corticosterone may be elevated for up to 1 week, but levels subsequently fall back towards control levels. Similarly, ACTH levels do not remain elevated in chronic stress paradigms, (Hashimoto *et al,* 1988), although in one chronic restraint stress paradigm, while ACTH levels fell with repeated exposure, elevated plasma corticosterone concentrations persisted over 16 days (Hauger *et al.*, 1990).

An important role for AVP in chronic or repeated stress is supported by a number of studies. It has been suggested that endogenous AVP is essential for sustaining the pituitary adrenal stress response in circumstances when the pituitary is refractory to CRF stimulation (Scaccianoce *et al.*, 1991). Chronic restraint stress also result in a loss of anterior pituitary CRF receptors, but this loss does not prevent a signficant potentiation of ACTH release with an alternative acute stress, suggesting that adaptation to repeated stress is stressor specific (Hauger *et al.*, 1990). Taken together, these data provide compelling evidence that AVP may be an important regulator of pituitary responsiveness in the face of chronic stress.

In summary, chronic/repeated stress can be characterized by generally normal circulating levels of ACTH and corticosterone. The system may, however, be more sensitive to alternative acute stressors, and there is evidence that the pituitaries of chronically stressed animals may be hypersensitive to the effects of AVP. Furthermore, adrenal weight is increased together with POMC mRNA and ACTH content of the anterior pituitary.

Mechanism of Action of CRF

In rats and primates CRF is a potent stimulus of ACTH secretion *in vivo* and *in vitro* (Vale *et al.*, 1983) as well as of POMC expression (Levin et al., 1991). A number of studies have demonstrated CRF binding sites in rat and primate pituitaries, with an affinity in the nanomolar range (Aguilera *et al.*, 1987) which is in agreement with the concentration of CRF required to stimulate ACTH release in isolated pituitary cells and the levels of CRF in pituitary portal circulation (Fink *et al*, 1988; Plotsky *et al.*, 1989).

The stimulatory effect of CRF on ACTH release is mediated by activation of adenylate cyclase and cAMP-dependent protein kinase (Abou-Samra *et al.*, 1987). In addition, there is evidence that non-cAMP mechanisms such as calcium and phospholipid metabolites are involved in postreceptor mediation of the effect of CRF (Abou-Samra *et al.*, 1987; Antoni, 1990). Differential regulation of these signaling systems by interaction with other regulators during chronic stress may play an important role in modulating the effect of CRF in corticotroph and pituitary ACTH responsiveness.

Regulation of Pituitary POMC Levels during Chronic Stress

The levels of POMC mRNA and ACTH content in the pituitary of chronically stressed rats vary according to the nature of the stimulus. In physical-psychological stress paradigms, which are usually associated with hypersensitivity of pituitary ACTH responses to novel stress, POMC mRNA levels are elevated. This has been shown following repeated footshock, swim stress, cold exposure, and i.p. hypertonic saline injections (Kiss & Aguilera, 1993; Young *et al*, 1993).The mechanism of the increased pituitary POMC mRNA levels during chronic stress is likely to involve stimulation by CRF. The 41-residue peptide has a marked stimulatory effect on POMC transcription, an effect which is mediated by cAMP (Lunnblad & Roberts, 1988).

Role of VP and CRF in the ACTH Response to Stress

The full ACTH response to acute stress depends on the release of both CRF and vasopresin VP as shown by studies using immunoneutralization or antagonists for these paptides (Guillaume *et al.*, 1992). Consistent with the participation of both CRF and VP, the raise in plasma ACTH in response to acute stress is associated to increases in amplitude and frequency of the peaks (Carnes *et al*, 1988). In chronic stress paradigms associated with corticotroph hyperresponsiveness show increases in VP expression in the parvocellular system, presumably resulting in increasing rations of VP : CRF secreted into the portal circulation. Therefore, increases in the proportional release of VP and its synergistic action of with CRF are likely to have a critical role in maintaining the responsiveness of HPA axis during repeated stress.

The participation of other regulators in addition to CRF and VP modulating corticotroph responsiveness during chronic stress cannot be excluded. A number of factors, such as angiotensin II, catecholamines, cytokines, have been shown to stimulate ACTH secretion in vitro or to potentate the stimulatory effect of CRF (Antoni, 1986).

Stress and the hypothalamic-pituitary-testicular axis

The effect of stress on the pituitary-testicular axis have attracted the attention of several authors for many years. It is well established that exposure to chronic psychological or somatic stress induced a drop in plasma testosterone (T) levels in several species (Rose *et al.*, 1972; Gray *et al.*, 1987) including man. Stress-related hormones can influence sexual functions at all three levels of the hypothalamic-pituitary-gonadal axis: the brain (to inhibit GnRH secretion),

the pituitary (to interfere with GnRH-induced LH release), and the gonads (to alter the stimulatory effect of gonadotropins on sex steroid secretion).

There are major differences in the response of the hypothalamic-pituitary-testicular axis to aversive stimuli depending on the species utilized and the type of stressor applied. Intact rodents (Mann *et al*, 1986; Turpen *et al*, 1976; Marić *et al*, 1991) exposed to an acute stress respond with a small and often short-lived increase in plasma LH and testosterone levels. The mechanisms responsible for the increased LH release are not well understood but one hypothesis concerns that stress activates hypothalamic noradrenergic neurons which in turn exert complex stimulatory and inhibitory effect on GnRH release (Marić *et al*, 1995).

Chronic stress on the other hand is constantly found to inhibit serum testosterone concentrations (Sapolsky, 1985; Tache *et al*., 1976; Marić *et al*., 1991). This decrease has generally been attributed to the suppresion of pituitary LH secretion and has been shown to be independent of adrenal secretions (Gray *et al*, 1978; Tache *et al*., 1980). However, several studies have recently shown that a decrease in plasma T levels may occur in acute stressed animals in the presence of normal circulating LH levels (Srivastava *et al*., 1993; Marić in press) indicating impared testiculair function primarly at the testicular level.

Stress-induced Changes in hypothalamic-pituitary axis

Stress decreases GnRH secretion, modulating effect of various pathways (in particular, those depend on CRF, endogenous opioid peptides, and/or biogenic amines) that are activated during stress and have impact on GnRH-secreting neurons.

One of the main factors recognized to exert a potent inhibitory influence on GnRH secretion is CRF, which acts as a neurotransmitter or neuromodulator in the CNS, and plays a role in the integration of an organism's response to stress (Rivier *et al*., 1985; 1986). However, the mechanisms through which CRF influences GnRH release are not fully understood and are likely to involve the activation of other pathways such as those dependent on endougenous opiates (Petraglia *et al*., 1986) and/or catecholamines (Valentino, 1989).

Role of the Endogenous Opioids

Hormonal responses to stress are mediated in part by endogenous opioid peptides (EOP): qualitatively the same responses can be elicited in the absence of stress by intracerebroventricular administration of opiod analogous (Halasz *et al*, 1981.), and stress-elicited responses can be modified (potentiated or blocked) by opioid antagonists or by passive immunoneutralization of EOPs (Petraglia *et al*, 1987.) However, the blockage by antagonists is often incomplete irrespective of dosage, showing that hormonal changes are only partially modulated by a central activation of EOP mechanisms. Endogenous opioids exert an inhibitory influence on LH secretion by acting on hypothalamic LHRH secretion (Cicero *et al*., 1985; Van Vugt *et al*., 1982). Various types of stress have been demonstrated to increase secretion of the endogenous opioids (Holtz et al., 1986; Harbuz & Lightman, 1989) suggesting that the endogenous opiates may mediate the effect of stress on gonadotropin secretion. In our laboratory an attempt was made at quantifying the relative contributions of opioid-mediated and opioid-independent components of the responses of prolactin (PRL), luteinzing hormone (LH), ant T levels to acute restraint stress. Our results demonstrated that the PRL reaction to stress is expected to be enhanced by two EOP-mediated mechanisms: by the activation of the PRF (prolactin releasing factor) pathway (Johnston *et al*, 1986.), and by the potentiation of the serotonine-mediated desinhibition of the dopamine-suppressed PRL secretion (Johnston & Negro-Vilar, 1986.). The stimulating nature of both the EOP-mediated and the EOP-independent components would explain the remarkable increase of PRL in acute stress. On the other hand the EOP-independent reaction to stress is expected to be opposed by two EOP-based and stress-enhanced inhibitory mechanisms: the potentiation of the serotonin mediated inhibition of LHRH release (Johnson & Crowley, 1984.), and the inhibition of its norepinephrine-

mediated stimulation (Adler & Crowley, 1984.). The mutual opposition of EOP-dependent and EOP-independent components of the reaction would explain the relatively small increase of LH (and T) observable during the acute phase of reastraint stress.

The effect of chronic stress on hypothalamic POMC derived peptides is controversal, although it cannot be exclude the fact that pituitary β-endorphin or other hypothalamic opioids play an important role in the stress-induced decrease in gonadotropin secretion.

Role of Biogenic Amines

Hovewer, the role played by catecholaminergic, serotoninergic, and cholinergic systems in the inhibition of GnRH neuronal activity during stress is less well known. The catecholaminergic system is highly activated during stress (Plotsky *et al.*, 1989) and there is extensive evidence for the involvement of catecholamines in the regulation of the HPA axis activity.

Monoaminergic-dependent pathways in the hypothalamus are belevied to mediate the antireporoductive effects of EOP (Gopalan *et al.*, 1989; Johnson *et al.*, 1986). In particular, systemic injection of morphine significantly decreases hypothalamic norepinephrine concentrations and plasma LH levels, but increases dopamine (DA) and serotonin (5-HT) activity in the hypothalamus (Gopalan *et al.*, 1989). During severe physical stress 5-HT could be involved in the decrease of GnRH neuronal acitivity. This hypothesis is supported by the observation that the 5-HT neuronal system from raphe nuclei to the hypothalamus is highly activated during stresses (Assenmacher *et al.*, 1987) and that 5-HT is able to inhibit GnRH secretion (Arias *et al.*, 1990). However, the precise role played by the serotoninergic pathways in the stress-indiced inhibiton of GnRH neuronal activity remains to be fully clarified.

Effect of stress on testicular level

As already ponted out stress lowers serum T values even in the presence of unchanged LH levels (Charpenet *et al*, 1981; Collu *et al.*, 1979; Srivastava *et al*, 1993). Such a dissociation between LH and testosterone values after exposure to stress led as to study the mechanism responsible for testosterone suppresion at the testicular level. According to our results (Marić *et al*, in press) as well as results from other laboratories (Srivastava *et al*, 1993; Akinbami *et al.*, 1994) can be postulated that acute stress imparied testicular steroidogenesis primarily at the testicular level, decreasing the activity of steroidogenic enzymes: P450 17α, lyase and 3β HSD since the inhibition of testosterone production after exposure to stress conditions were not associated with concomitant reduction in number of LH receptors (Tache *et al.*, 1980) or testicular hCG binding (Orr *et al.*, 1994). In the light of these findings coupled with present result it should be consider that the effect of acute stress is mediated at a post LH receptor site, i.e. the steroidogenic function of the Leydig cells. According to the our results as well as the results from other laboratories it might be consider that acute stress impairs T production mostly affecting through a combination of the local and the central effects. Since the effect of chronic stress is mainly pronounced on the hypothalamic-pituitary level.

The effect of stress on reproductive functions and the mechanisms mediating these effects depend on type, duration, and frequency of the stimulus (Collu *et al.*, 1984). In particular there is evidence that „the response of the pituitary-testicular system to aversive stimuli is potentially biphasic, with an initial stimulatory phase and, if stress is prolonged or of sufficient magnitude, a subsequent inhibitory phase" (Gray *et al.*, 1978). The mechanisms mediating the stress response of the hypothalamic-pituitary-testicular axis are mediated at the level of GnRH-secreting neurons, while long-term effects also involve peripheral mechanisms such as alteration in pituitary and gonadal responsiveness. There is also little doubt that though the early effect of stress may, at least under some circumstances, be stimulatory, prolonged or severe stimuli are accompanied by booth an increased activity of the HPA axis and a suppresion of reproductive functions. Because of the complexity of the interactions involved in the activity of GnRH neurons, a good understanding of the precise central mechanism that mediate the antireproductive effects of stress is still lacking.

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EFEKTI STRESA NA NEUROENDOKRINU REGULACIJU

Pregledni članak

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U priloženom preglednom članku dat je prikaz odgovora neuroendokrine regulacije na stresogone stimuluse. Prvo je opisana reakcija hipotalamo-hipofizne-adrenalne (HPA) osovine na akutni i hronični stres, kao primarne za adaptaciju organizma. Drugi deo rada obuhvata prikaz efekata akutnog i horničnog stresa na hipotalamo-hipofizo testikularnu osovinu a što dovodi do opadanja reproduktivne funkcije u stresu.

Aktivacija HPA osovine u stresu izaziva naglo povećavanje ACTH u cirkulaciji sa odgovarajućim porastom glikokortikoida a što je neophodno za uspešnu adaptaciju. Porast ACTH u stresu je regulisan hipotalamičnim regulatorima, tj. proporcionalan je sekreciji CRF i VP u praventrikularnom nukleusu hipotalamusa, modulacijom hipofiznih VP receptora i negativnom povratnom spregom glikokortikoida.

Efekti stresa na hipotalamo-hipofizo tekstikularu osovinu se manifestuju poremećajem reproduktivne funkcije usled smanjene koncentracije testosterona u cikrulaciji. Smanjena biosinteza testosterona je posledica delovanja stresogenih stimulusa na sva tri nivoa hipotalamo-hipofizo testikularne osovine. U hroničnom stresu smanjen nivo testosterona je primarno uslovljen delovanjem stresogenih stimulusa na centralnom nivou. Centralni mehanizmi koji su odgovorni za antireproduktivne efekte su kompleksni i uključuju promene u sekreciji i interakciji neurotransmitera, endogenih opioida kao i međuzavisnosti hipotalamo-hipofizo testikularne osovine i hipotalamo-hipofizo adrenalne osovine. U akutnom stresu dolazi do značajnog poremećaja u lokalnoj parakrinoj autoregulaciji testisa. Smanjivanje koncentracije testosterona u prisustvu normalne sekrecije LH je posledica aktunih stresogenih stimulusa na steroidogenezu testisa usled smanjivanja aktivnosti steroidogenih enzima.

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Pregledni članak

THE EFFECT OF CAPTAN ON REPRODUCTION, TERATOGENICITY AND CARCINOGENICITY IN SOME SPECIES OF MAMMALS

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INTRODUCTION

Captan, a chloroalkyl thio heterocyclic fungicide (N-/trihlormetiltio/1,2,5,6, tetrahidroftalimid) has been evaluated by the Joint Meetings on Pesticide Residues in 1965, 1969, 1977, 1978, 1982, 1983, 1984, 1986 by FAO/WHO.

The studies of captan on reproduction, teratogenicity and carcinogenicity in some species of mammals gave fairly contradictor results. But one fact become clear, that a full acceptable daily intake (ADI) dose of 0.1 mg/kg body weight was established on the basis of the no-effect level (NEL). The latter studies included the investigations of dose-related effect of captan in acute (short-term) and chronic (long-term) treatment, applied on one or more generations. Data from an additional study complemented and extended earlier results, establishing that captan level does not cause toxicological effect at 12,5 mg/kg body weight/day for rat, as well as for rabbit, 120 mg/kg body weight/day for mouse and 50 mg/kg body weight/day for hamster (1,2). These studies have become available and are summarized and discussed in this monograph addendum.

TOXICOLOGICAL STUDIES

Studies on reproduction

The first evidence of toxicological effects of captan on reproduction has been done from Aldrige et al. (3). Charles River CD strain female and male rats were fed with captan in the diet at dosages of 6,12,5, and 25/mg/kg body weight/day for 102 days. After the treatment the animals were mated. Fertility index, length of gestation and pup viability and weight at birth were comparable to controls. Goldenthal (4), folowed three-generation of Charles River CD strain female and male rats. They were fed with captan in the diet at the equivalent of 25, 100, 250 and 500 mg/kg body weight/day for 102 days and then mated. A reduction in pregnancy was observed at the dosages of 250 and 500 mg/kg body weight/day. In the progeny generation litter size at birth was reduced in all treated groups in F_1 and F_2 generation litters at 100 mg/kg

body weight/day. Pup survival was adversely affected at 250 mg/kg body weiht/day in F_1, F_2 and F_3 litters throughout lactation period. The same author (5) found that the number of corpora lutea per dam was significantly reduced already with dosage at 25 mg/kg body weight/day for 102 days. The effect of captan on reproductive processes in three generation of rats (Wistar strain, both sexes) we used and treated with two NEL dose: 3.5 and 7.5 mg, daily per animal for 113 days (6). The results we obtained showed that the chronical treatment of captan caused considarable changes in the estrous cucle, characterized by a prolongation of the proestrous phase, from one to 2–3 days in 65% of the females of all three generations. The presence of polyfolicular, cystic ovaries, observed in 46.6% ot the animals, indicate that some mature de Graff's folicules did not burst but were converted into ovarial cysts which latter fused into a single macrocyst. Significant reduction of estradiol level in the serum, particularly in F_2 generation, indicates to reduction the number of corpora lutea in the ovaries, which is in accordance with Goldenthal (5). With higher dose of captan the number of ova was reduced for 59.69%. In F_1 generation 42.86% and in F_2 28.57% of the animals did not ovulate. Some of gravide females had less number of descendants according to controls. Reduction of number of ova (the consequence is less number of corpora lutea), high percentage od anovulated females and less number of descendants, could be explain by decreasing of luteinizing hormone (LH) amount released from the adenohypophysis.

These studies demontrate a withdrow of the previous opinion that captan have no effect on reproduction, and sugests that chronic application of captan causes distturbances in reproductive processes in the female rats.

Studies on teratogenicity

According to the results reported by Caurtney et al. (7), pregnant CD-1 mice, exposed via inhalation to captan at approximately 488 mg/hour/m^3, 4 hours/day, produced no malformed foetuses. Similarly, captan was not teratogenic in pregnant CD-1 mice treated with 100 mg/kg body weight/day of the compound orally or subcutaneously. Reduction of maternal body weight and a slight increase in foetal mortality were noted.

In a pilot teratology studies with mated female Golden Syrian hamsters given oraly doses of captan at 50, 100, 200, 300 and 400 mg/kg body weight/day, only adverse effect observed was a dose-releated decrease in maternal weight gain at 300 mg/kg body weight/day (8). In the next experiment, following the same dosage, the same author (8) found depression of maternal and foetal weight, at 200 mg/kg body weight/day. In general, the incidence of malformations appeared to be comparable in all groups. However, the incidence of non-specified rib anomalies in foetuses and litters was at higher dose. These bent rib anomalies may be accounted by the maternal stress effect upon the foetus rather than a teratogenic effect (10).

Orally application of captan in mated female New Zealand white rabbits at 6, 12, 25 and 60 mg/kg body weight/day, increased incidence of non-specific signs (anorexia, reduction of feacal output and water consumption), litter and foetal weights were depressed with dose at 60 mg/kg body weight/day (11).

Groups of 30-day-old Charled River CD rats (males and females) were administered with captan in the diet at dose levels of 25, 100 and 250 mg/kg body weight/day for two years (9). The results showed that the mean body weight for males and females given 100 and 250 mg/kg body weight/day were significatnly lower than a controls. Aldrige et al. (3) found slight reduction in mean pup body weight and litter weight on lactation days at 25 mg/kg body weight/day of captan. With captan at dose 3.5 and 7.5 mg/per animal/day, for 113 days (6), we did not find any difference of the body weight according to the controls. In some animals, of both sexes, a bulk of the stomach was couspicously enlaged. Depilation with erosions of the skin, particularly in the region of the neck and inguens, has been evident in some number of animals. Study of dermal penetration of captan at dosage of 0.2, 0.5 and 2.7 mmol/cm^3 for 72

hours (12) was found to increase as dosage decreased and the penetration valuess in young rats (33-day-old) washigher than in adults (82-day-old). Described dermal penetration of captan and our findings of erosions of the skin could be explain by a idea that, by blood flow system through subdermal capillary net, ensure the apsorption and disposition of captan. The data that captan and captafol are immunogens, as hapten, bound to some serum protein (13), could provoke also, insted of immune response, the allergy and erosions of the skin are possible the epidermal injury, peculiar for the second phase of allergy.

Studies of carcinogenicity

Studies of carcinogenicity of captan was done in some species of mammals. The incidence of tumours and lesions were investigated by Gross postmortem macroscopic and microscopic examinations.

Feeding the female and male, 35-day-old, mice with 8.000 and 16.000 ppm of captan for 80 weeks provoked adenomatous polyp and polypoid adenocarcinoma of the duodenum, but incidence was not statisticaly significant, exept in females at 16.000 ppm dose (14). Using a different dose of captan, at 2.000, 6.000, 10.000 and 16.000 ppm Bradfield et al, (15) discover the presence of duodenal lesions and gestrointestilal adenomas and adenocarcinomas, particularly with mid- and high-dose. The results demonstrate that captan, at dietary concentration of 10.000 and 16.000 ppm induced both benign and malignant duodenal tumours in the mice. An increase of the incidence of duodenal tumours was observed by bioassay at dietary levels of captan ranging from 6.000 to 16.000 ppm (16). According to the report of histopatological findings by Arceo (17) the lesions in the duodenum were present.

There is evidence that even lower dose (800 and 6.000 ppm) increased incidence of duodenal hyperplastic lesions, the benign and malignant duodenal neoplasias, respectively. Increased incidence of lesions of the small intestine (masses, nodules, raised areas) were revealed (18).

Groups of 30-day-old Charles River CD rats, both sexes, were administered captan in the diet of dose levels of 25, 100 and 250 mg/kg body weight/day for two years. Microscopically, hepatocellular hypertrophy was significantly increased in high-dose animals only. Male rats demonstrated a statistically significant trend for kidney adenomas (5). In our studies of long-term treatment with captan (6) by macroscopic examination of internal organs we did not observed statistically significant neoplasias of duodenum, but in some cases proliferation of duodenal mucotic tissue, specially Bulbus duodeni, was evident. Although, the occurence of unilateral or bilateral tumours on kidney was present, but we did not assess microscopicaly a type of tumours. The carcinogenic potency of captan was analyzed using a medium-term multi-organ bioassay (19) for carcinogenicity. Male F 344 rats were treated during a period od 20 weeks. Neoplastic and pre-neoplastic lesions were found in the thyroid and kidney. Also, the number of glutathione-S-transferase placental-form-positive liver cell foci was significantly increased.

Summarizing a data, an ability of carcinogenic effect of captan, in long-term treatment, on some organ tissues is evident.

CONCLUSION

This study of captan represents a rewiev of its effect on reproduction, teratogenicity and carcinogenicity in some species of mammals. In data of FAO/WHO 1982, 1984 was established that no-effect level (NEL) dose of captan for rat is 12.5 mg/kg body weight/day, as well as for rabbit, 120 mg/kg body weight/day for mouse and 50 mg/kg body weight for hamster.

The studies of toxical effects of captan on reproduction are evident. A reduction in pregnancy was observed with higher as well as with lower dose, by chronic application. Considerable changes in the estrous cycle, presence of polyfolicular cystic ovaries and reduction the number of corpora lutea in the ovaries could be explained by decreasing amount of luteinizing hormone (LH) released from adenohypophysis. This data sugests that captan causes a disturbance in reproductive proceses.

Oraly application of captan over NEL dose slightly depressed maternal, foetal and litters body weight. Teratogenic study in hamster registrates the incidence of non-specific malformation of rib in foetuses and litters. In some treated rats a bulk of the stomach was conspicuously enlarged. Depilation with erosions of the skin has been evident in many cases. In could be explained by data that captan is immunogen and could provokes also the allergy.

Studies of carcinogenic effect of captan were investigated by Gross postmortem macroscopic and microscopic examinations. The incidence od duodenal hyperplastic lesions, the benign and malignant duodenal neoplasias were increased in mice, rats and hamster. Increased incidence of lesions of the small intestine in the mice was evident. Unilateral and bilateral tumours on the kidney, neoplastic lesions in the thyroid gland and increased the number of glutation-positive liver cell foci were revealed. An ability of carcinogenic effect of captan, in long-germ treatment, on some organ tissues is evident.

In conclusion, based on these studies, there is confirmation that captan, in long-term treatment can disturbs reproductive processes, have slight teratogenic effect and could be carcinogen.

EFEKAT KAPTANA NA REPRODUKCIJU, TERATOGENOST I KARCINOGENOST KOD NEKIH VRSTA SISARA

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U ovom radu dat je pregled delovanja kaptana na reprodukciju, teratogenost i karcinogenost kod nekih vrsta sisara. Na osnovu podataka FAO/WHO, od 1982 i 1983. utvrđeno je da je doza bez efekta (NEL) kaptana za pacova 12.5 mg/kg telesne mase/dnevno, kao i za zeca, 120 mg/kg telesne mase/dnevno za miša i 50 mg/kg telesne mase/dnevno za hrčka.

Istraživanja su pokazala da kaptan ima uticaja na reprodukciju. Redukcija graviditeta zapažena je kako sa višim tako i sa nižim dozama (u odnosu na NEL) kaptana, hronično primenjenog. Značajne promene u estrusnom ciklusu, prisustvo polifolikularnih, cističnih ovarijuma i redukcija broj žutih tela u ovarijumima mogla bi se objasniti smanjenjem količine luteinizujućeg hormona (LH) otpuštenog iz adenohipofize. Ovi podaci sugerišu da kaptan izaziva poremećaje reproduktivnih procesa.

Oralna primena kaptana iznad NEL doze izaziva neznatno smanjenje telesne mase kod majke, fetusa i novorođenčadi. Teratogeni efekat kaptana bio je izražen kod hrčka u vidu nespecifičnih malformacija rebara kod fetusa i novorođenčadi. Kod izvesnog broja tretiranih pacova masa želuca je bila vidno uvećana. U mnogo slučajeva bila je evidentna depilacija sa erozijama kože, što bi se moglo objasniti podatkom da je kaptan imunogen, a samim tim bi mogao prouzrokovati i alergiju.

Izučavanja karcinogenog efekta kaptana vršena su makroskopskim i mikroskopskim pregledom, nakon žrtvovanja životinja. Pojava duodenalnih hiperplazija, benignih i malignih duodenalnih neuplazija povećana je kod miševa, pacova i hrčkova. Takođe je evidentirano povećanje lezija na tankom crevu kod miševa. Kod pacova je konstatovano prisustvo unilateralnih i bilateralnih tumora na bubrezima, neoplastične lezije tireoidnih žlezda i povećanje

glutation-pozitivnih fokusa ćelija jetre. Prema tome, evidentno je postojanje karcinogenog efekta kaptana, dugotrajno primenjivanog, na tkiva nekih organa.

Da zaključimo, na osnovu ovih izučavanja može se konstatovati da dugotrajna primena kaptana može izazvati poremećaje u reproduktivnim procesima, ima manji teratogeni efekat i mogao bi biti karcinogen.

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Pregledni članak

ULTRASTRUKTURNE ODLIKE NEUROSEKRETORNIH ĆELIJA U MOZGU Lumbricus rubellus Hoffm. U STRESU

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ABSTRAKT

Proučavane su ultrastrukturne odlike nneurosekretornih ćelija u mozgu Lumbricida / Lumbricus rubellus Hoffm./ u uslovima stresa. U dve ogledne grupe životinja proučavani su uticaji triju istih agresivnih faktora spoljašnje sredine: povišene temperature, pojačane vlažnosti i gladovanja. Pored navedenih faktora kišne gliste su bile po grupama izlagane kontinuiranom uticaju svetlosti i uticaju tame. Prema ovim podacima obe grupe životinja su bile u stannju hroničnog stresa. Ultrastrukturne odlike neurosekretornih ćelija u životinja izlaganih kontinuiranoj svetlosti podrazumevaju hipertrofiju Golgi-eve zone, obilnije prisustvo endoplazmatskog retikulma, slobodno raspoređenih ribozoma. Ovo govori u prilog stimulisane sinteze. Posebno se ističu promene u građi mitohondrija i pojava lamelarnih formacija. O pojačanoj metaboličkoj aktivnosti govori i povećan broj lizozoma u ćelijama. Proces vakuolizacije citoplazme i prisustvo brojnih lamelarnih tela čine bitne odlike ćelija u proučavanom stresu. Reagovanje neurosekretornnih ćelija pod uticajem svetlosti je progresivnog karaktera. U životinja izloženih kontinuiranoj tami neurosekretorne ćelije odražavaju vid smanjene aktivnosti kako na sintezu tako i na mobilizaciju neurosekreta, tako da su reaktivne promene u neurosekretornim ćelijama depresivnog karaktera. Mastociti kao poseban vid ćelija, koje pripadaju međuprostornom tkivu, pratioci su gradivnih elemenata mozga i neurosekretorne su prirode.

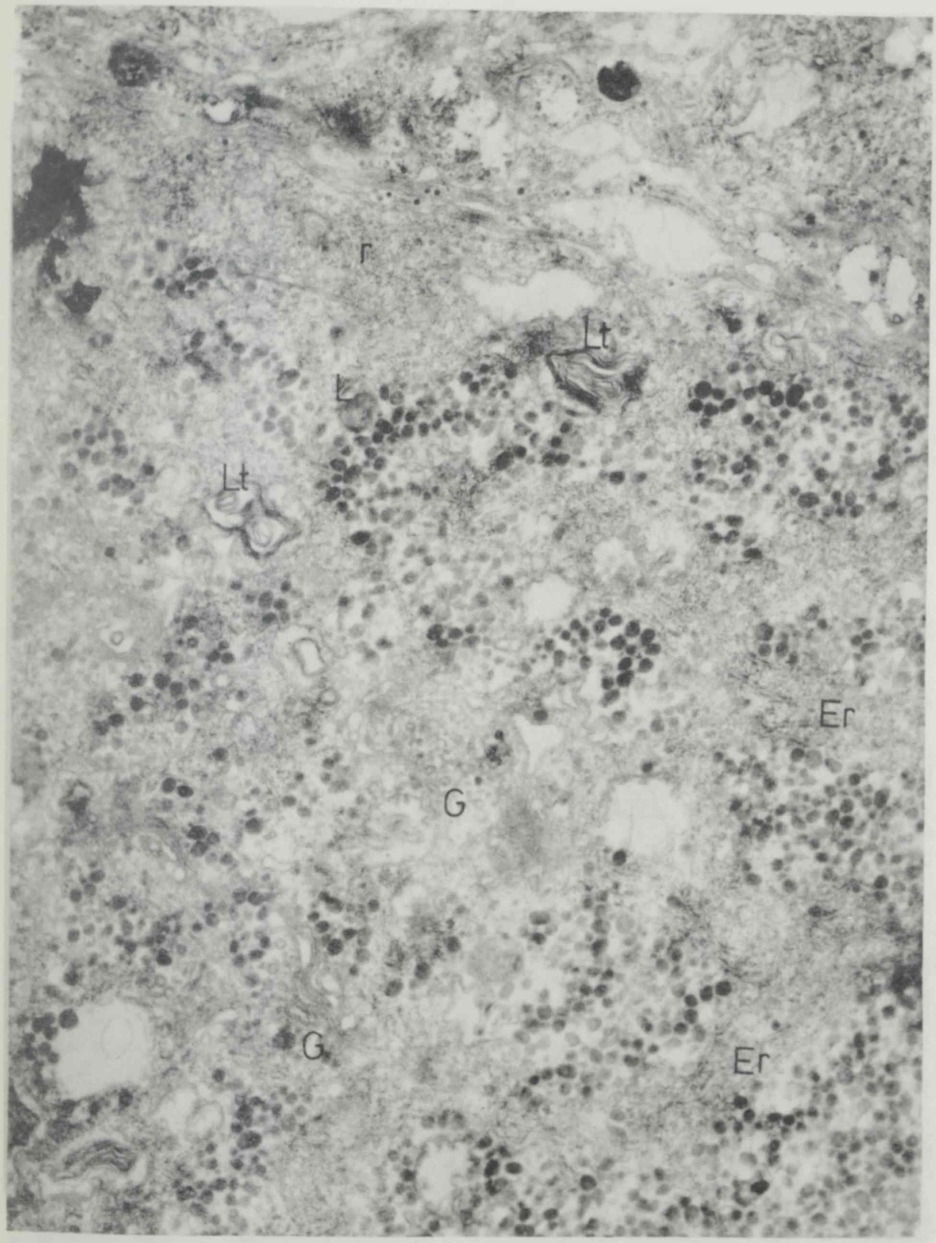
Ključne reči – Neurosekretorne ćelije, mozak, ultrastruktura, Lumbricidae, neurosekrecija, mastociti

UVOD

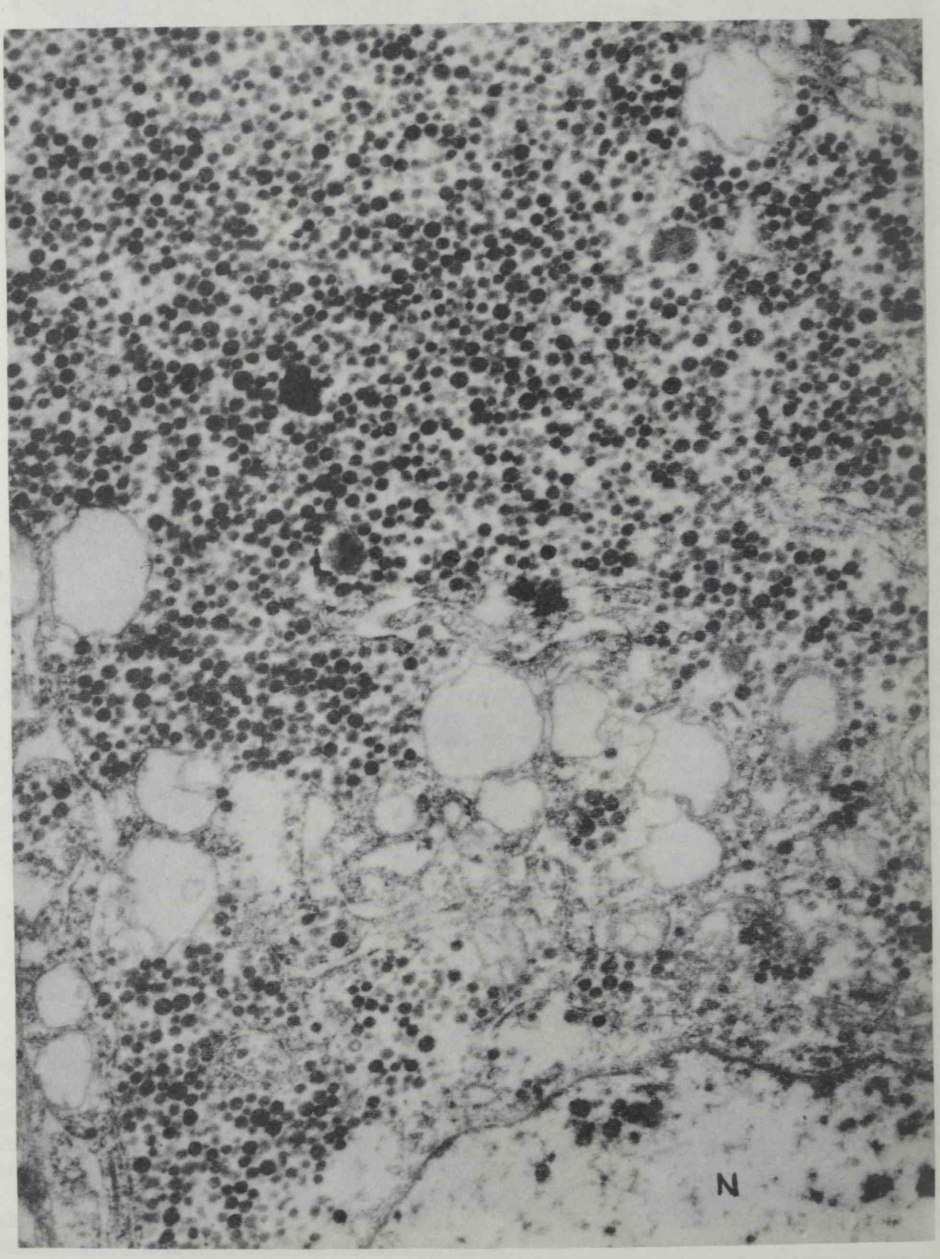
Jedan od najaktuelnijih problema neuroendokrinologije danas predstavlja neuroendokrina funkcija mozga. Neurosekretorne ćelije u mozgu kontrolišu kako rast i razvoj tako i mnoge druge funkcije organizma, pa je stoga i razumljivo veliki značaj koji se pridaje istraživanju njihove morfologije i fiziologije. Sve do skoro nije bilo dovoljno podataka o endokrinoj aktivnosti velikog broja vrsta beskičmenjaka. Brojnim proučavanjima danas su sakupljeni podaci koji svedoče da je endokrina aktivnost veoma razvijena i zastupljena od Cnidaria pa sve do svih viših oblika životinja.

Iako je veliki broj autora obrađivao fenomen neurosekrecije još uvek se malo zna o udelu neurosekretornih produkata u regulaciji pojedinnih aktivnosti organizama kao i o biloškim svojstvima neurosekreta u beskičmenjaka.

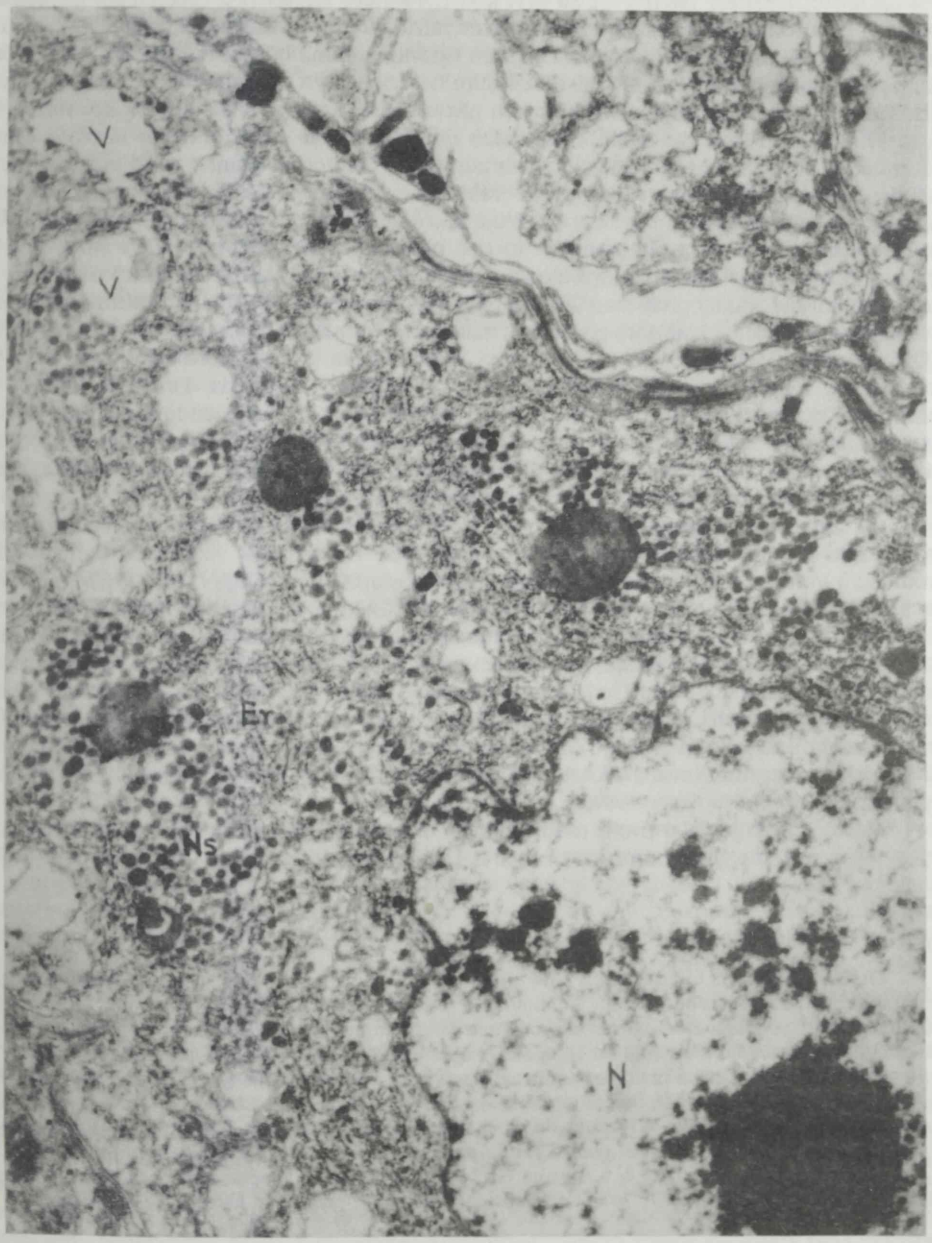
Prvi radovi koji se odnose na istraživanja nervnog sistema u Lumbricida sa metodama elektronnske mikroskopije su Scharrer i Brown 1961, Röhlich idr. 1962, Oosaki 1966, Levi i dr. 1966, Berjon i Meunier 1968, Stevanović 1976, 1977, 1978, 1978a, 1979, 1979a, Stevanović i Matavulj 1988, Csoknya 1992, Al-Yousuf 1992, 1992a, Stevanović i Karaman 1994.



Sl. 1. NEUROSEKTRETORNA ĆELIJA TIPA I
ogledna grupa: uticaj svetlosti
G – Golgi-eva zona; Lt – lamelarna tela; Er – granulirani endoplazmatski retikulum;
r – ribozomi; L – lizozomi; 20.000 x



Sl. 2. NEUROSEKRETORNA ĆELIJA TIPA II
ogledna grupa: uticaj svetlosti
Vakuole i cisterne granuliranog endoplazmatskog retikuluma; N – jedro; L – lizozomi; 20.000 x



Sl. 3.NEUROSEKRETORNA ĆELIJA TIPA III
ogledna grupa: uticaj svetlosti
N – jedro; Nl – nukleolus; Er – enndoplazmatski retikulum;
Ns – granule neurosekreta; V – vakuole; L – lizozomi; 20.000 x

Scharrer i Brown 1961i 1962 su prvi kod Lumbricus terrestris u cerebralnoj gangliji sa nalazima elektronske mikroskopije opisali važne infrastrukturne razlike, od ćelija do ćelije. Te razlike su pripisivali različito opisanim ćelijskim tipovima prema opštim histološkim karakteristikama. Ovi autori smatraju da je reč o različitim funkcionalnim stanjima jednog istog ćelijskog tipa. Röhlich i dr. 1962 na ultrastrukutrnom nivou u cerebralnoj fangliji kišne gliste razlikuju tri različita vida neurosekretornih ćelija. Jedan tip ćelija prema ovim autorima nazvane ćelije akumulacije odlikuju se izobiljem elementarnih neurosekretornih granula koje mogu zauzeti veći deo ćelijskog tela. Oosaki 1966 u cerebralnoj gangliji kišne gliste Eisenia foetida nalazima elektronske mikroskopije opisuje nervne ćelije kao šest tipova neurona koji sadrže morfološki različite inkluzije. Ovaj autor opisuje četiri tipa neurona sa neurosekretornimn svojstvima u pravom smislu, a dva tipa ćelija opisuje kao „obične" neurone. Na ultrastrukturnom nivou u našim ranijim radovima opisali smo u mozgu kišne gliste Lumbricus rebellus Hoffm. Četiri tipa neurosekretornih ćelija koje smo poredili sa nalazima svetlosne mikroskopije /Stevanović 1978, 1979/. Berjon i Meunier 1968 u cerebralnoj gangliji kišne gliste u kortikalnoj zoni koja je heterogenog izgleda razlikuju tri ćelijska tipa bez posebnog rasporeda. Levi i dr 1966 su u centralnom nervnom sistemu Lumbricus trerrestris opisali da su forme citoplazmatičnih inkluzija zajedničke za glijalne ćelije i neurone i da je materijal sličnog tipa bio prisutan ekstracelularno u neuralnoj lameli. Zimmermann 1968 opisuje kompleks „ćelija" u centralnom nervnom sistemu Lumbricus terrestris kojima pripisuje svojstvo mastocita.

Otremba 1961 je ispitivao na Lumbricus terrestris neurosekretornne promene izazvane promenama životne sredine. Ovaj autor je ustanovio, pri različitoj vlažnosti i temperaturi, promene u kvantativnom sadržaju pojedinih ćelijskih grupa. Aros i Vigh /1961, 1962/ su opisali uticaj eksperimentalnih delovanja na neurosekretorni sistem u kišnih glista. Massiat 1965 je na kišnoj glisti Allobophora chlorotica ispitivala kombinovano uticaj svetlosti sa i bez infracrvene komponente. Cadariu 1966 je opisala promene neurosekretornih ćelija izazvane raznim eksperimentalnim uticajima u vrste Octalasium lacteum. Svetlost a naročito vlažnost prema ovom autoru izazivaju hipersekreciju koja vodi do pražnjenja neurosekretnog materijala iz ćelija preko aksona i na holokrini način. Mnogi autori su opisivali neurosekretorne ćelije u mozgu kod različitih vrsta Lumbricida Oosaki 1966, Berjon i Meunier 1968, Al-Yousuf 1992, 1992a, Lyckman i dr 1992, Benvolgy 1994.

Namera nam je da u ovom radu opišemo ultrastrukturnne promene neurosekretornih ćelija u mozgu kišne gliste Lumbricus rubellus Hoff. u stresu.

MATERIJAL I METODE

Objekat ispitivanja je kišna glista Lumbricus rubellus Hoffm. Životinje su bile izlagane stresogenim faktorima u dve grupe: uticaju kontinuirane svetlosti jačine 6000 lux, pojačanoj vlažnnosti i gladovanju. Druga grupa životinja je bila izložena kontinuiranoj tami, pojačanoj vlažnosti, povišenoj temperaturi i gladovanju u trajanju od 14 dana. Temperatura je iznosila 20^0C, a vlažnost 85%.

Prednji deo životinje s prvih pet segmenata, stavljen je u puferov 5% gluteraldehidni fiksativ /0,2 m Na-cacodylat puferu pH 7,4/. Smeša je sadržala i 5% saharozu. Vreme fiksacije je 3,5 časa na sobnoj temperaturi. U ovom fiksativu je i vršena disekcija mozga za dalji postupak. Pranje je izvršeno dva puta po 10 minuta u 0.2 M Na-cacodylat puferu pH 7,4 sa 3% saharoze. Mozak je zatim fiksiran u 1% OsO_4/ 0.2 M Na-cacodylat puferu pH 7,4/ u trajanju od 2,5 sata na sobnnoj temperaturi. Dehidratacija mozga izvršena je u seriji alkohola 50%, 70%, 96%, apsolutni alkohol i propilen a kalupljenje u epon 812.

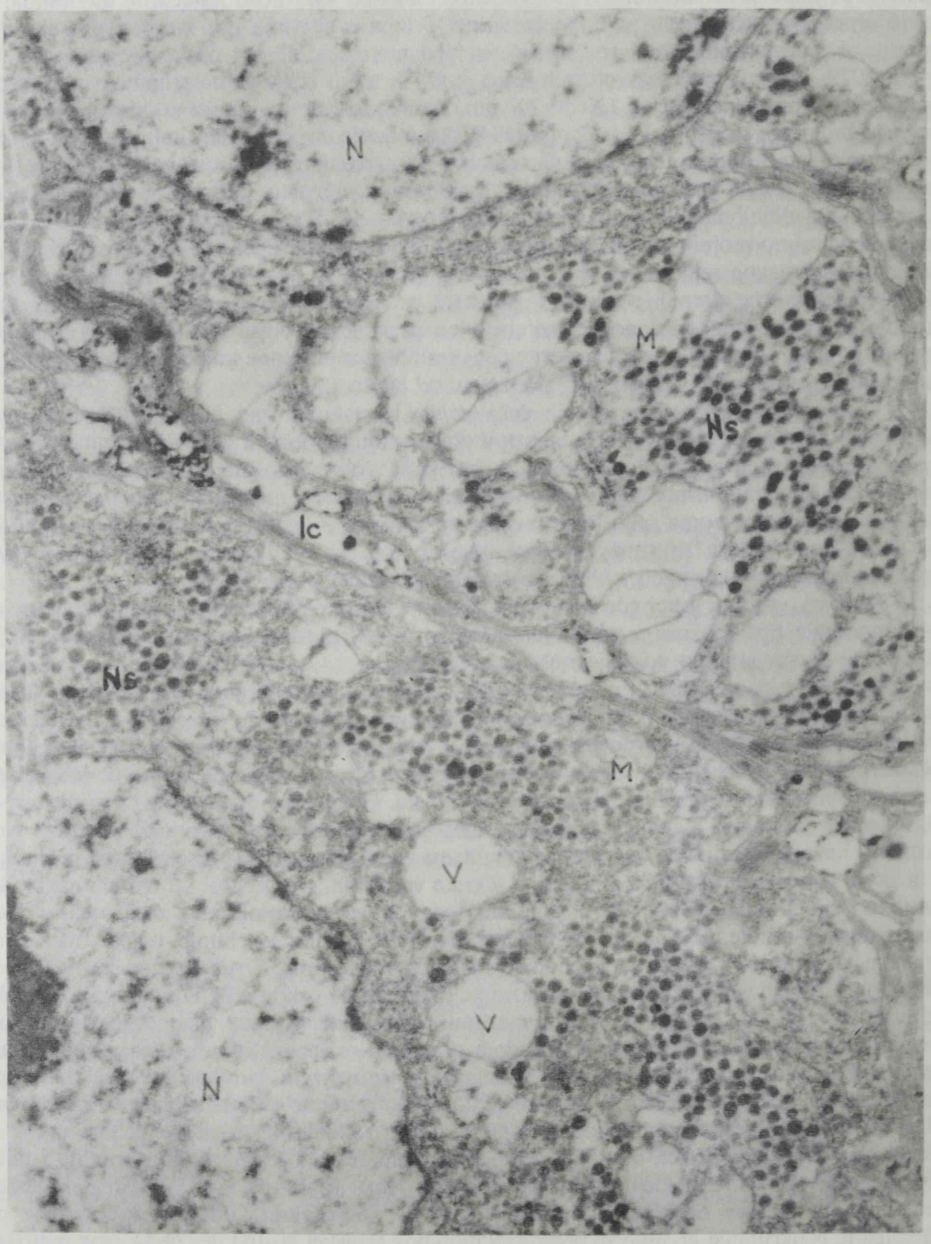
REZULTATI

Ultrastrukturne odlike neurosekretornih ćelija u mozgu Lumbricus rubellus Hoffm. se odlikuju velikom varijabilnošću u veličini, oblikovanju i intezitetu neurosekretnih granula.

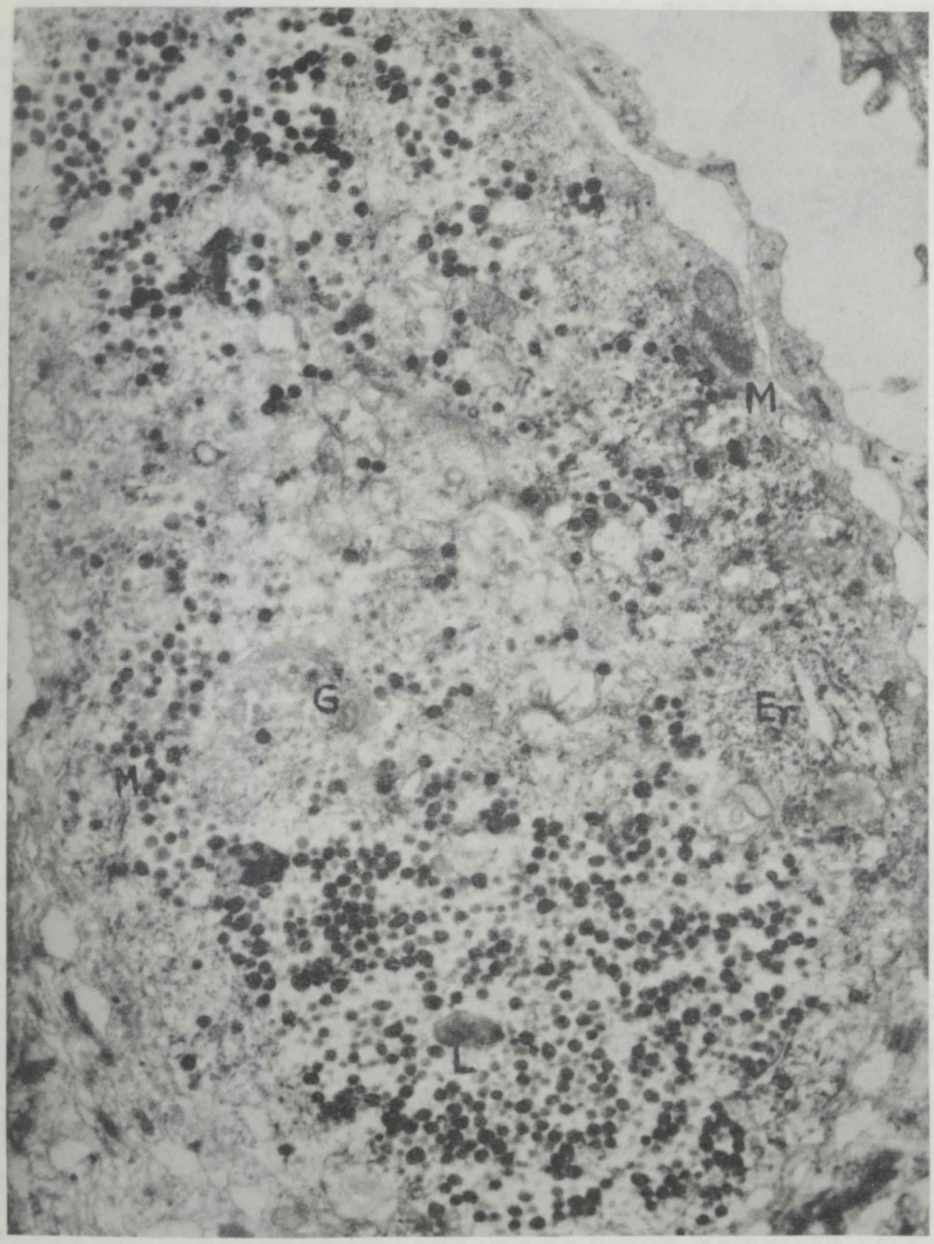
Jedra ovih ćelija su sa mestimičnim ulegnućima odnosno valovitosti nuklearne membrane sa jasno omeđenim prostorom podjednake širine. Prema svojstvima pre svega neurosekretnih granula u mozgu Lumbricida srećemo četiri tipa neurosekretornih ćelija /Stevanović 1977, 1978, 1979/. Prvi tip neurosekretornih ćelija je tip I, to su ćelije sa najkrupnijim granulama neurosekreta prosečne veličine 120 do 200 nm. Neurosekretorne granule su difuzo raspoređene veće brojnosti, sferičnog ili ovalnog oblika, dosta neujednačenog denziteta, sa ređe izraženom osmiofilnom opnom. Deo granula se nalazi u intercelularnom prostoru. Ćelije tipa II su sa granulama menjih promera veličine 100 do 130 nm u prečniku. Granule su neujednačenog denziteta, pojedine opasane osmiofilnim membranama koje naležu na matriks granule. U ovim ćelijama nalazimo brojnije zastupljene ribozome slobodno raspoređene u citoplazmi. Membrane endoplazmatskog retikuluma su granuliranog svojstva. U neurosekretornim ćelijama tipa III neurosekretorne granule su manjih promera prosečne veličine od 60 do 120 nm. Ove granule su dosta neujednačenog denziteta. Neurosekretorne ćelije tipa IV su sa najvećim brojem granula opasanih sa svetlijim oreolom osmiofilne membrane koja je prostorno odvojena od sadržaja granule. Promeri ovih granula su od 80 do 130 nm.

U citoplazmi neurosekretornnih ćelija sreću se vakuole kao i koncentrično sazdana lamelarna tela. Srećemo veliku varijabilnost u oblikovanju mitohondrija kao i načinu prostiranja mitohondrijalnih kresti. Pojedine mitohondrije su dobro ispoljene građe. Pojedine mitohondrije su ispoljenije osmiofilije i valovitosti mitohonndrijalne membrane. Golgi-eva zona se odlikuje prisustvom veoma spljoštenih vrećica, varijabilnog su položaja u citoplazmi. U blizini Golgi-eve zone nalaze se sitna osmifilna telašca koja su udaljavanjem povećavanju svoj volumen uporedo sa sticanjem oblika svojstvenim sekretornim granulama.

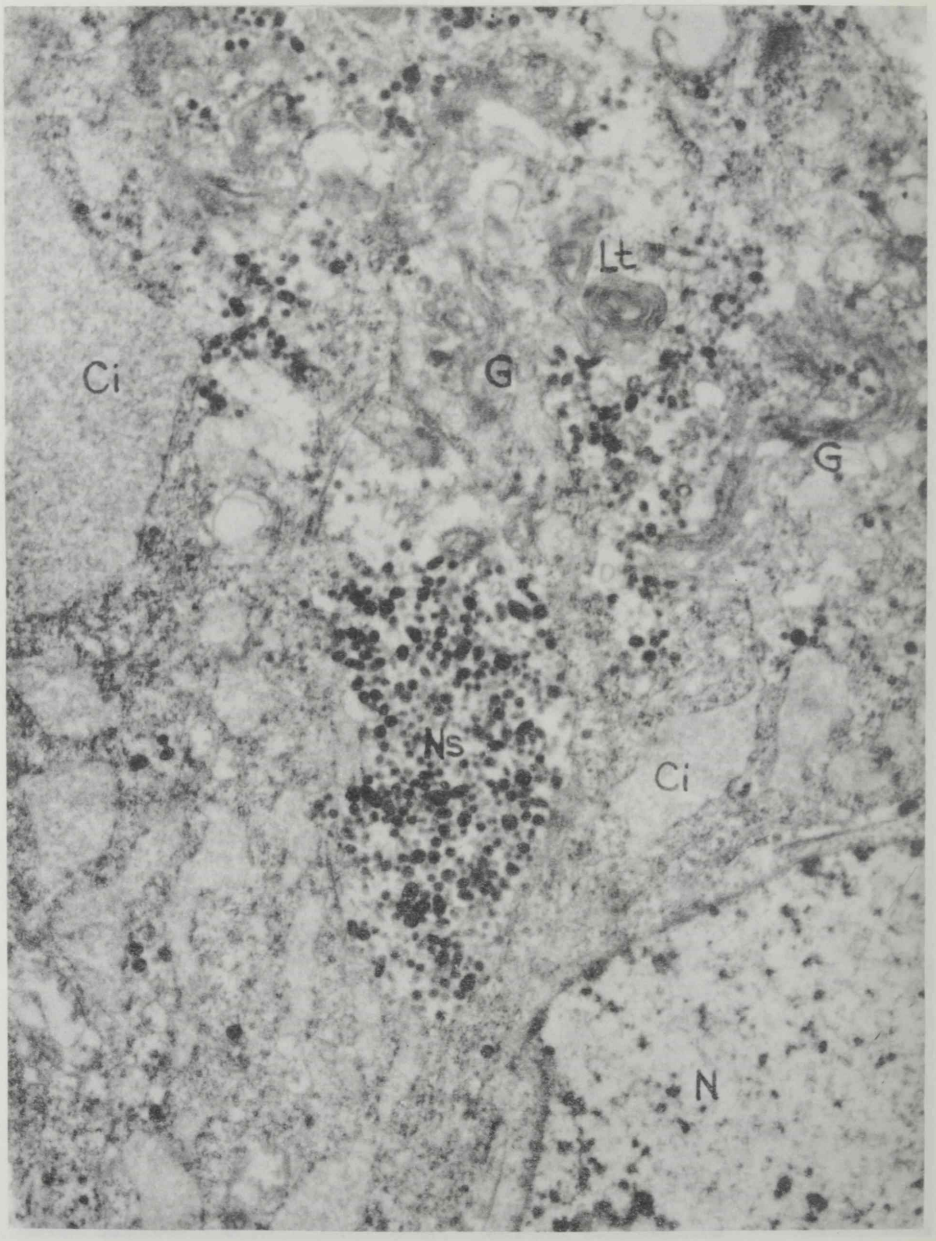
U prvoj oglednoj grupi gde su životije iclaganje kontinuiranom uticaju svetlosti neurosekretorne ćelije tipa I ukazuju na veoma izražene ultrastrukturne reaktivne promene. Sl. 1. Neurosekretorne granule su nejednako raspoređene u citoplazmi. Granule su grupisane, pretežno su sferičnog oblika. Endoplazmatski retikulum je granuliranog tipa relativno široko rasprostranjen. Izraženo je veće prisustvo slobodno raspoređenih ribozoma u citoplazmi. Mitohonndrije su veoma polimorfne. Posebno je karakteristična intermitohondrijalna geneza lamelarnih tela čije brojnije prisustvo u citoplazmi ćelija tipa I u prve ogledne grupe predstavlja jednu od specifičnih reaktivnih ultrastrukturnih odlika. Golgi-eva zona je šireg prostranstva. U blizini sastavnih delova zone nalaze se osmiofilne grannule koje postepenim povećanjem svog promera i svojim dezitetom odgovaraju granulama neurosekreta. Neurosekretorne ćelije tipa II u prvoj oglednoj grupi su sa granulama sekreta varijabilnog prisustva, od manjih nagomilavanja u pojedinim delovima citoplazme do toga da su neurosekretna zrna znatno ređe zastupljena. Granule sekreta su varijabilnog denziteta. Jednu od bitnih odlika ovih ćelija predstavlja proširenje grauliranog endoplazmatskog retikuluma. Drugu specifičnu odliku u ultrastrukturi ovih ćelija predstavlja oblikovanje enndoplazmatskog retikuluma poput vakuole, odnosno cisterni. (sl. 2) Unutar cisterni, mestimično isprekidanog zida, ustanovljeno je prisustvo granula neurosekreta. Mnoštvo slobodnih ribozoma se zapaža oko nuklearne membrane kao i slobodno raspoređeni ribozomi u citoplazmi u vidu rozete. Neurosekretorne ćelije tipa III su sa velikim jedrom često režnjevite građe. Oko jedra se nalaze brojnije koncentrično raspoređene membrane graulrianog endoplazmatskog retikuluma. Granule neurosekreta se zapažaju u vidu aglomerata opasnih endoplazmatskim retikulumom, ali ima granula i difuznnog rasporeda u perinuklearnom ili perifernom delu citoplazme. (sl. 3) Neurosekretorne ćelije tipa IV u prve ogledne grupe sadrže granule neurosekreta difuzno raspoređene po citoplazmi. Najkrupnije granule su veoma osmiofilnog svojstva gustog denziteta svog matriksa sa uskim svetlim pojasom nejednake širine i slabije ispoljenom osmiofilnom membranom. Postoje granule manjih dimenzija od predhodnih, smajene osmiofilije, bez omotača. U ovim ćelijama zapaža se obilje endoplazminog retikuluma sa paralelno usmerenim mebranama uz prisustvo brojnih ribozoma. (Sl. 4.)



Sl. 4. NEUROSEKRETORNA ĆELIJA TIPA IV
ogledna grupa: uticaj svetlosti
Er – granulirali endoplazmatski retikulum; V – vakuole;
Ns – Granule neurosekreta; 35.000 x



Sl. 5. NEUROSEKRETORNA ĆELIJA TIPA I
ogledna grupa: uticaj tame
Er – granulirani endoplazmatski retikulum; G – Golgieva zona;
M – mitohondrija; L – lizozom; 20.000 x



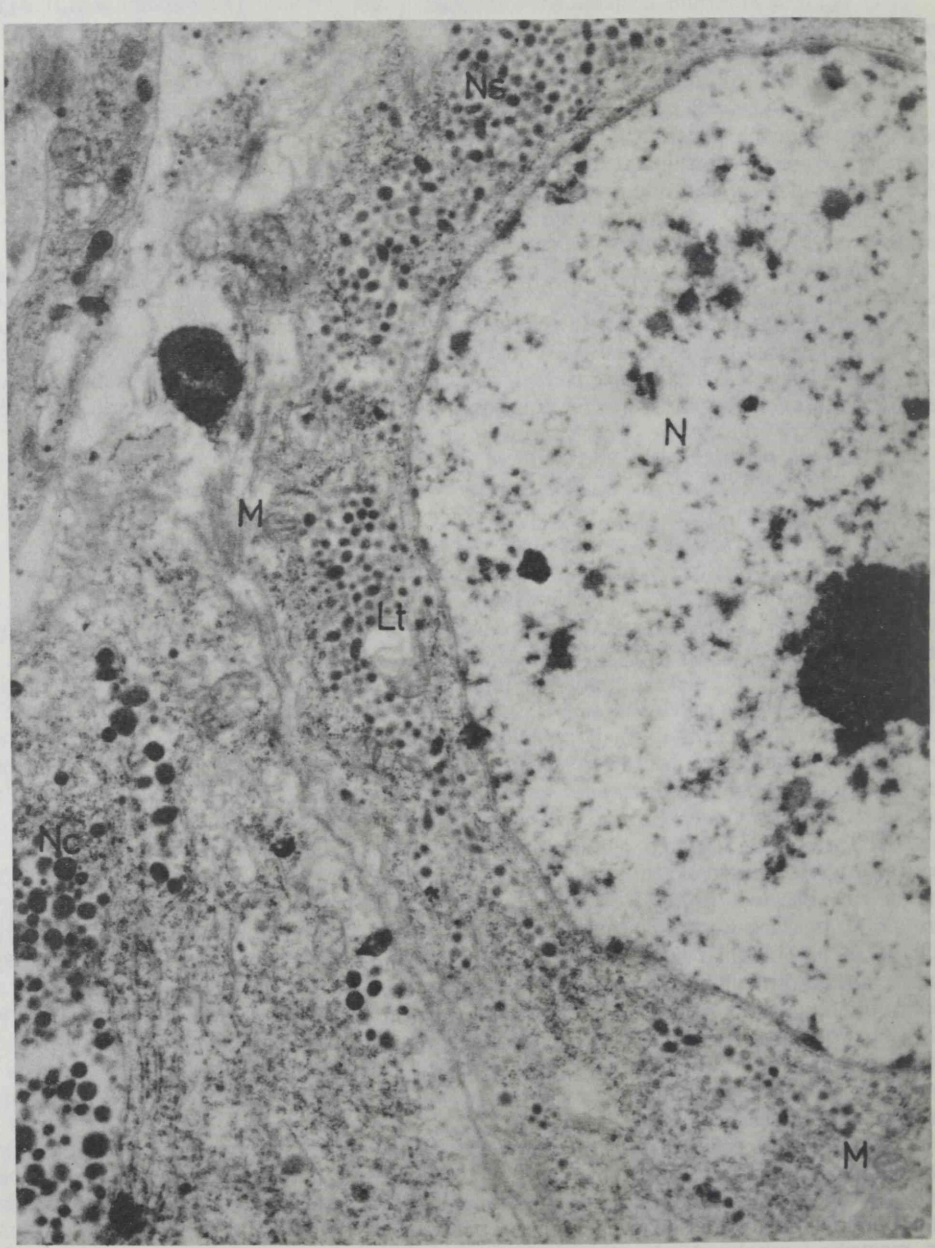
Sl. 6. NEUROSEKRETORNA ĆELIJA TIPA II
ogledna grupa: uticaj tame
N – jedro; Ns – neurosekretorne granule; Ci – cisterne;
G – Golgi-eva zona; Lt – lamelarna tela; 20.000 x

U neurosekretornim ćelijama prve ogledne grupe mitohondrije su veoma varijabilne građe i veličine. Pored mitohondrija sa dobro očuvanim krestama i dvostrukom mebranom, ima ih u kojima se zapaža razjedinjavanje mitohondrijalnih kresti, do proširenja matriksa koji gubi svoj denzitet, dolazi do postepenog formiranja vakuola i do geneze, u lumeu vakuola, ili u neposrednoj blizii njihovog zida lamelarnih tvorevina veoma varijabilne građe. Postoje lamelarne tvorevine kocentrično izgrađene, lamele u vidu prstena ili drugih tvorevina. U pojedinim vakuolama razlikuju se vilozni izdannci njihovog zida, osmiofilnog svojstva. U citoplazmi ovih ćelija nalazimo prisustvo tamnih krupnih tela sferičnog oblika, jasno ispoljenog graničnog pojasa, ali i tela koja su neravnije površine, nejasnijih granica, svetlijeg denziteta od predhodnih. Prva tela bi odgovarala lizozomima a druga lipidnim kapljicama.

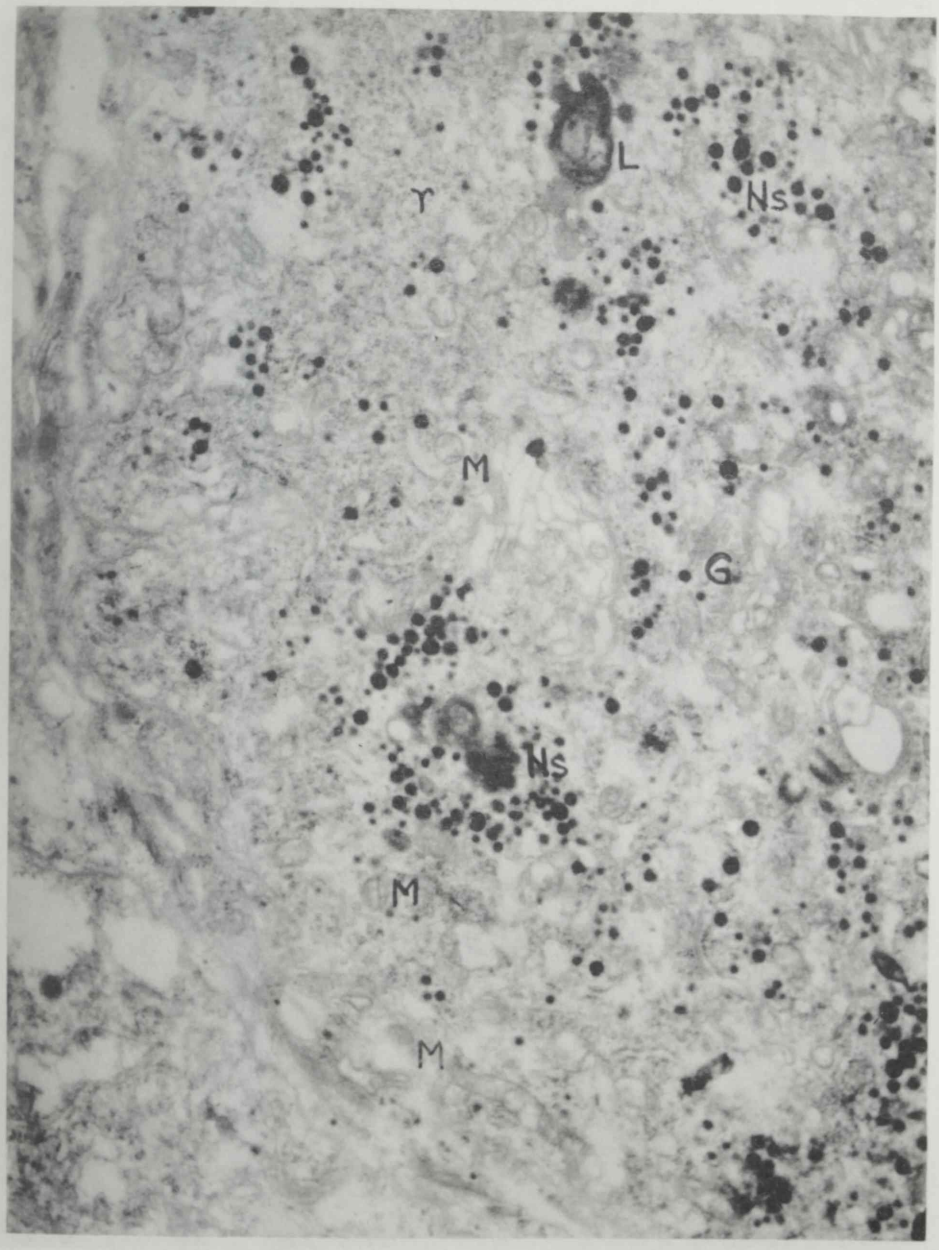
U mozgu Lumbricida srećno posebno diferencirane ćelije koje svojim ultrastrukturnim odlikama se predstavljaju kao mastociti. Mastociti se pružaju duž neuroglandularnih perikariona kao i njihovih aksona. Mastocite srećemo i u mrežolikom tkanju neuropila. Ultrastrukturna odlika mastocita bila bi prisustvo krupnih granula koje su u promeru do 400 nm. Granule su pretežno ovalnog oblika neujednačenog rasporeda. U pojedinim mastocitima ustanovljeno je prisustvo kristaloidnnih figura. Sa površinne ćelijskog tela odvajaju se mikrovilozni izdaci nejednake širine i dužine.

U drugoj oglednoj grupi kod životinja izložennih kontinuiranoj tami nneurosekretorne ćelije tipa I se odlikuju nepravilno raspoređenim granulama. Ima neurosekretornih granula u vidu manjih ili većih grupa ali i u vidu prostranijih aglomerata. Granule su uglavnom gustog denziteta. Ispoljava se manje prisustvo ribozoma. Golgi-eva zona je suženog prostranstva, delimično suženih vrećica i oskudnnijim brojem vakuola u svom sastavu. (Sl. 5.) Ćelije tipa II se odlikuju gomilicama neurosekreta. Drugu bitnu ultrastrukturnu odliku ovih ćelija čini prisustvo cisterni u sastavu granuliranog endoplazmatskog retikuluma ispunjennim veoma sitnim sadržajem. (Sl. 6.) Granični pojas cisterni je u vidu membrane čija je osmiofilija mestimično smanjenna ili diskontinuirana, tako da dolazi do međusobne komunikacije cisterni. Granule neurosekreta u ćelijama tipa III su varijabilnog denziteta. Njihov raspored je perinuklearno ili u vidu većih skupina. U pojedinim ćelijama suženog prostranstva citoplazme, oko perinuklearne membrane nalazi se prostor u vidu diskontinuiranog prstena omeđen sa spoljašnje strane membranom granuliranog endoplazmatskog retikuluma /sl. 7./. U tom prostoru se nalaze razbacana zrna ribozoma i slabije prisustvo neurosekreta. Jedra su krupna jasno omeđena sa difuzno raspoređenim hromatinom, a jedarce je bobičave strukture hiperosmiofilnog svojstva. U neurosekretornim ćelijama tipa IV u životinnja izloženih tami granule su varijabilne veličine i osmiofilije postavljene u manjim grupama ili pojedinačno. Pojedine granule su opasane svetlim pojasom između matriksa granule i osmiofilne membrane. Zastupljenni su granulirani i glatki endoplazmatski retikulum. Pojedine ćelije ovoga tipa imaju znatno slabiju osmiofiliju neurosekretornih granula. Pojedine ćelije svetlog sadržaja opasane su dobro diferenciranom osmiofilnom mebranom. Metohondrije su u neurosekretnim ćelijama u životinja izloženih tami znatno očuvanije građe mada se u znatno manjoj meri ispoljava fenomen intramitohondrijalnih lamelarnih tela. Mitohondrije su uglavnom različite veličine i oblika često izdužennog tubuloznog vida sa znacima mestimične vakuolizacije i geneze sitnih lamelarnih diferencijacija (sl. 8.).

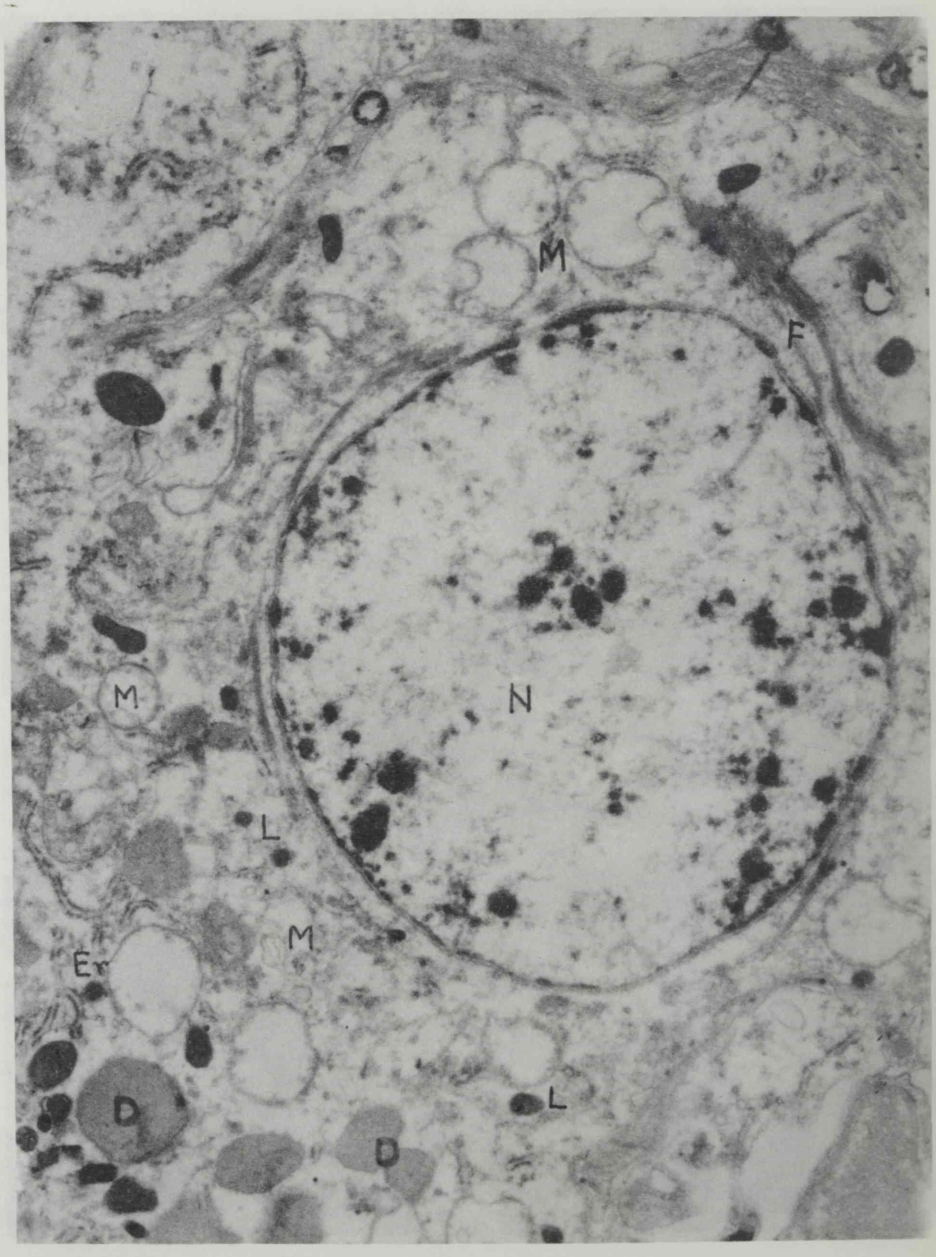
Neuroglijske ćelije u mozgu Lumbricida se odlikuju pravilijim oblicima jedra, dobro ispoljennim perinuklearnim prostorom kao i porama. Mitohondrije su veoma varijabilnnog izgleda. Uočavaju se sa dobro očuvanom građom ali i sa povećanim volumenom, nestajanjem mitohondrijalnih kresti i ispoljavaje figura sferičnog oblika. Uočavaju se mitohondrije u kojima se maatriks i mitohondrijalne kreste slivaju u jednu masu, s postepenim razdvajanjem i nestajanjem mitohondrijalnne membrane i pretvaranjem u tamna tela. Golgi-eva zona je razvijenije strukture nego u neurosekretornim ćelijama i većeg je prostranstva. Neuroglijske ćelije sa svojim produžecima sadrže endoplazmatski retikulum granuliranog tipa mrežoliko isprepleten između ostalih ćelijskih organela. Jednu od bitnih odlika neuroglijskih ćelija pred-



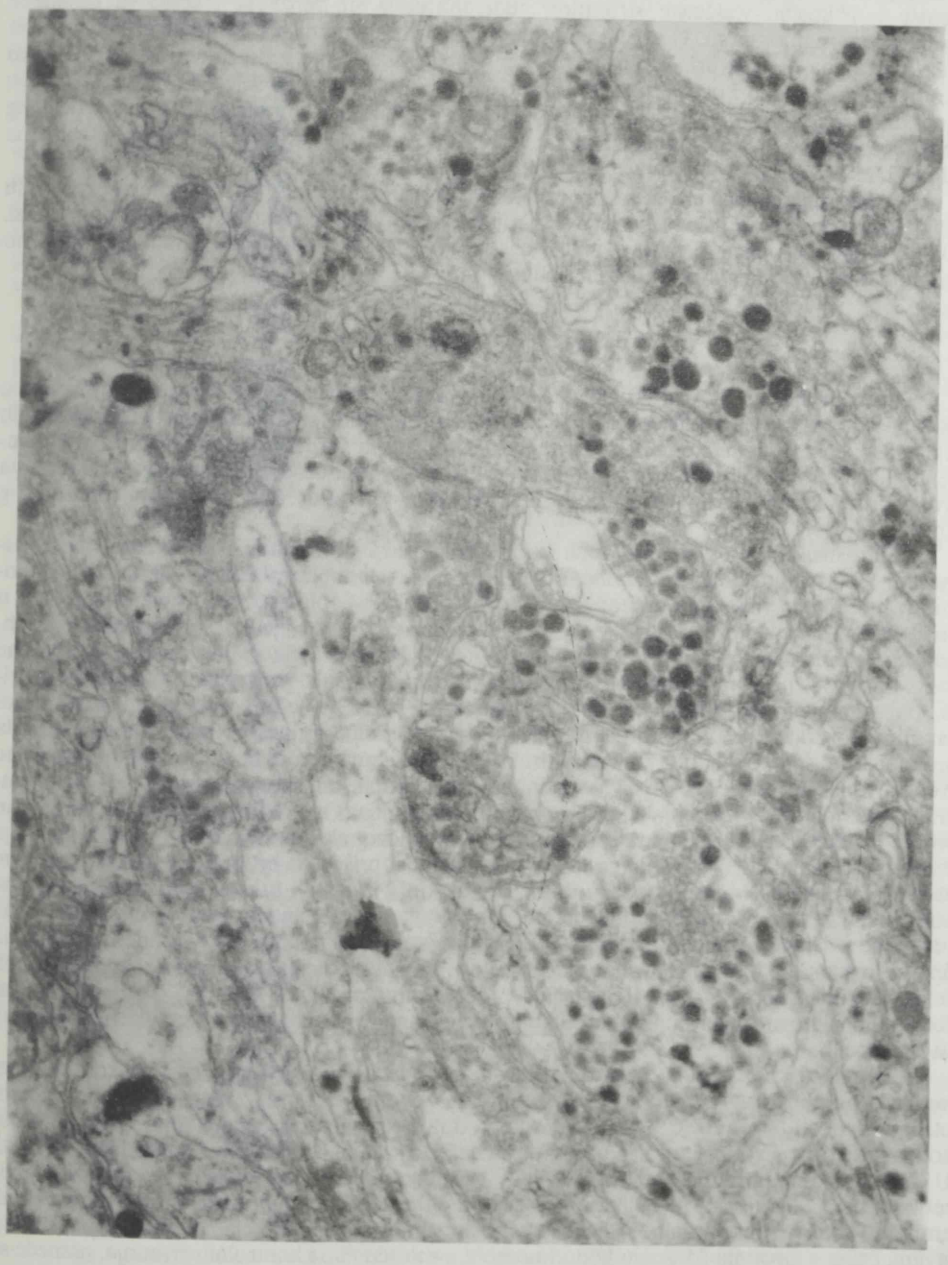
Sl. 7. NEUROSEKRETORNA ĆELIJA TIPA III
ogledna grupa: uticaj tame
N – jedro; Nl – nukeolus; Ns – granule neurosekreta;
M – mitohondrija; Lt – lamelarna tela; Nc – neurosekretorna ćelija tipa I; 20.000x



Sl. 8. NEUROSEKRETORNA ĆELIJA TIPA IV
ogledna grupa: uticaj tame
Ns – granule neurosekreta; M – mitohondrija; r – ribozomi;
G – Golgi-eva zona; L – lizozom; 20.000 x



Sl. 9. NEUROGLIJSKA ĆELIJA
N – jedro; M – mitohondrija; Er – granulirani endoplazmatski retikulum;
D – tamna tela; F – glija vlakna; L – lizozomi; 20.000 x



Sl. 10. PODRUČJE NEUROPILA
Prošireni delovi aksona neurosekretornih ćelija;
u njima granule neurosekreta i mikrovezikule; 35.000 x

stavlja prisustvo tamnih tela nejednako raspoređenih u citoplazmi /sl. 9./. Ova tela su sitnozrnaste i delimično vlaknaste strukture, neujednačenog su denziteta i često su omeđena osmiofilnom membranom. U pojedinim neuroglijskim ćelijama nalaze se multivezikularna tela dobro ispoljena u strukturi. Brojni produžeci neuroglijskih ćelija protežu se i isprepliću kako oko perikariona, tako i oko aksonskih produžetaka eurosekretornih ćelija. U blizini Golgi-eve zone kao i između tamnih tela uočava se prisustvo osmiofilnih tvorevina sferičnog ili ovalnog oblika, varijabilne veličine, pojedinačno ili grupno raspoređene koje bi po svojim osobinama odgovarale lizomima.

U području neuropila uočava se prisustvo proširenih delova aksona neurosekretornih ćelija, kao i proširenja u vidu butona sa varijabilnim sadržajem neurosekretornih granula /sl. 10./. U njima se nalaze i gomile mikrovezikula svetlog sadržaja, dobro omeđene, pretežno grupno raspoređene uglavnom u zoni sinapsi.

DISKUSIJA I ZAKLJUČCI

Proučavane su ultrastrukture odlike neurosekretornih ćelija u mozgu Lumbricida u uslovima stresa. U ove ogledne grupe životinja proučavani su uticaji triju istih agresivnih faktora spoljašnje sredine: povišena temperatura, pojačana vlažnost i gladovanje. Pored navedenih faktora kišne gliste su bile izlagane po grupama konntinuranom uticaju svetlosti a druga grupa uticaju tame. Prema ovim podacima obe grupe životinja bile su u stanju hroničnog stresa.

Ultrastrukturne odlike ćelija tipa I u životinja izlaganim kontinuiranoj svetlosti podrazumevaju hipertrofiju Golgi-eve zonne, obilnije prisustvo endoplazmatskog retikuluma i slobodno raspoređenih ribozoma u citoplazmi. Ovo predstavlja citofiziološke testove koji govore u prilog stimulisane sinteze. U Golgievoj zoni gde dolazi do masovnije geneze neurosekretornih, odnosno elementarnih granula. Varijabilni raspored i denzitet ovih granula govori u prilog ne samo njihove geneze već i njihovog sazrevanja. Posebno se ističe promena u građi mitohondrija i pojava lamelarnnih formacija, odnosno lamelarnih tela. Ovo je pojava koja se u kičmenjaka zapaža u pojedinim endokrinim žlezdama u procesima stimulisane produkcije hormona reda proteina odnosno polipeptida /Ziegels i dr. 1976/. U ćelijama tipa II pod uticajem kontinuirane svetlosti ističe se povećano prisustvo granuliranog endoplazmatskog retikuluma u vidu membrana, vakuolarnih proširenja u cisterni granuliranog zida. Ove odlike ukazuju na stimulisan proces sinteze u ovim ćelijama. O pojačanoj metaboličkoj aktivnosti govori i povećanje broja lizozoma u ćelijama. Proces vakuolizacije citoplazme i prisustvo brojnih lamelarnih tela čine najbitnije odlike u reagovanju ćelija tipa III u životinja izlaganih uticaju svetlosti u proučavanom stresu. Veliki polimorfizam elementarnih granula i varijabilnost njihovog denziteta, kao i smanjeno prisustvo granula opasanih svetlim pojasom i osmiofilnom memabranom u ćelijama tipa IV u životinja izlaganih kontinuranoj svetlosti pružaju indiciju o aktivnom reagovanju ovih ćelija u proučavanom stresu. Reagovanja progresivnog karaktera srećemo i u neuroglijskim ćelijama a njihovo reagovanje je korelativno reagovanju opisanih neuroglandularnih ćelija čiji su oni sateliti.

Ultrastrukturne odlike neurosekretornih ćelija tipa I u životinja izlaganih kontinuranoj tami znatno odudaraju svojim svojstvima od ćelija opisanih u životinja izlaganih kontinuiranom uticaju svetlosti /Stevanović 1978, 1979/. Upoređujući sa ćelijskim organelama u odgovarajuće kontrolne grupe životinja stiče se utisak da su ćelije ovoga tipa sa znacima smirenije aktivnosti. U prilog ovome ukazuju odlike Golgi-eve zone, kao i opisana svojstva endoplazmatskog retikuluma. Ćelije tipa II se odlikuju prisustvom cisterni, smanjenim brojem neurosekretornih granula i intermitohondrijalnom genezom polimorfnih lamelarnih tela. U poređenju sa svojstvima ćelija u životinja izloženih kontinuiranom svetlošću kao i kontrolnih životinja, nameće se pitanje da li su ove neurosekretorne ćelije u stadijumu smirenije aktivnosti. Na osnovu citodinamike ovih ćelija stiče se ovaj utisak u poređenju sa predhodno opisanim ćelijama. Sudeći prema svojstvima organela u neurosekretornim ćelijama tipa II, one bi bile odraz

ćelijskog stanja smirenije sinteze i eliminacije neurosekreta. U ćelijama tipa III u životinja izloženih tami najbitniju odliku čini pojava lamelarnih formacija, a u ćelijama tipa IV vakuolizacija motohodrijalnih kresti. Na osnovu poređenja ova poslednja dva tipa ćelija sa ophođenjem istovetnih ćelija u kontrolne grupe životinja, moglo bi se izvesti predpostavka da i one održavaju vid smanjene aktivnosti bilo u odnosu na sintezu bilo u odnosu na mobilizaciju, to jest izlučivanje neurosekretornih grannula.

Neuroglijske ćelije u životinja izloženih tami se ističu sa povećanim prisustvom tamnih tela, pojačanim denzitetom mitohondrija i dobro zastupljenim endoplazmatičnim retikulumom. Ustanovljeno je takođe prisustvo mladih nedovoljno diferenciranih ćelija koje bi odgovarale ćelijama – izvorima za obnovu neurosekretornih ćelija. Vršeći konfrontaciju ovih ćelija sa dinamikom neuroglijskih ćelija, stiče se utisak njihovog korelativnog reagovanja u uslovima proučavanog stresa. Reaktivne promene u neurosekretornim neuronima u životinja pod uticajem tame su depresivnog karaktera.

Mastociti kao poseban vid ćelija neuroglandularne prirode i njihovo prisustvo u mozgu Lumbricida, predstavlja interesantan podatak s obzirom na fiziološka svojstva ovih ćelija koje pripadaju međuprostornom tkivu. Ovaj vid ćelija opisali smo prvi put pod ekspermentalnim uslovima pod uticajima stresogenih faktora u Lumbricus rubellus /Stevanović 1978, 1979/. Sudeći prema našim nalazima predpostavljamo da su mastociti, kao poseban vid ćelija, pratioci gradivnih elemenata mozga neuroglandularne prirode. Od značaja su biloška svojstva mastocita posebno njihov biohemizam: sadržaj u heparinu, 5-hidroksi triptaminu, histaminu i izobilju enzima među kojima se naročito ističe prisustvo onih koji sudeluju u prometu belančevina i fosfatnih supstanci u međuprostornom tkivu /Keler 1962, Arvy 1968/. Imajući u vidu sva ova svojstva mastocita, moglo bi se predpostaviti da je njihov udeo u reaktivnim promenama neurosekretornih neurona u stanju stresa od posebnog značaja.

Imajući u vidu bilogiju kišne gliste posebno sezonskog ritma u manifestacijama aktivnosti mozga /Hubl 1953, Cadariu 1966, 1967, Al-Yousuf 1992, 1992a, Banvolgyi 1994/ smatrali smo da je proučavaje udruženih agresivnih faktora: povećane vlažnosti, povišene temperature, gladovanja i kontinuiranog uticaja svetlosti u jedne grupe odnosno uticaja tame u druge eksperimentalne grupe najprikladniji vid pristupa za bolje upoznavaje citodinamike, odnosno histofiziologije neurosekretornih ćelija u Lumbricida. Sa stanovišta stresologije moglo bi se postaviti pitanje da li bi svaki od proučavanih agresivnih faktora imao istovetno dejstvo kao njihov zbir. Na temelju rezultata koje smo dobili predpostavljamo da su ti rezultati odraz sadejstva svih stresogenih faktora proučavanih u eksperimentima koje smo izvodili. Postavlja se pitanje da li bi na temelju rezultata naših ispitivanja mogla predpsotaviti i druga funkcionalna svojstva neurosekretornih ćelija. Pošto nismo vršili sistematsku analizu reaktivnih promena u drugim delovima nervnog sistema u Lumbricida, mišljenja samo da na osnovu rezultata koje smo dobili proističe neminovnost njihove interpretacije sa stanovišta teorije o sindromu adaptacije /Selye 1950, 1951/. Po ovoj teoriji, u uslovima stresa prvenstveno su angažovani mehanizmi u cilju prilagođavanja, odnosno otpornosti ka agresiji. Sve oglednne životinje su bile u stanju hroničnog stresa pri čemu su eksperimentalno izmenjeni uslovi osmoregulacije usled povišenog stepena vlažnosti i spoljašnje temperature sredine. Mehanizam adaptacije kišne gliste na izmenjene uslove spoljašnje sredine u oglednih grupa podrazumevao je reaktivne promene opisane u neurosekretornim ćelijama u mozgu. Na temelju tih podataka predpostavljamo da su neurosekretorne ćelije mesto lučenja aktivnih principa odnosno neurohormona od presudnnog značaja u fenomenu adaptacije na izmenjene uslove spoljašnje sredine.

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ULTRASTRUCTURAL CHARACTERISTICS OF BRAIN NEUROSECRETORY CELLS IN STRESS AFFECTED LUMBRICUS RUBELLUS HOFFM

D. Stevanović, M. Matavulj. Z. Banovački

Summary

The effect of three equally assaulted environmental factors, i. e. starvation, increased temperature, and increased humidity was analyzed in two earthworm groups. Also, these animal groups were exposed to continnuous light and dark effects inducing a chronic stress.

In the earthworms under contiuous illumination, the Golgi-zone hypertrophy, abundance of endoplasmic reticulum, and freely arranged ribosomes in cytoplasm are the characteristics found in typel I cells. Such cell appearannce suggests a stimulated synthesis in the Golgi – zone where a more expressed genesis of neurosecretory granules occurs. Variaable arrangement and density of these granules implicate not only their genesis, but, also, their maturation. Changes in the mitochondrial strucutre and the occurrence of lamelar formations, namely lamelar bodies are worth noting. In type ll cells, continuous light resulted in a greater abundance of membranous granular endoplasmic reticulum, vacuolar enlargements, and cisternae with granulated walls. The above characteristics point to a stimulated synthesis in these cells. An increased lysosome number in cells also points to an ennhanced metabolic activity. Cytoplasmic vacuolation and the occurrence of numerous lamelar bodies are the most conspicuous characteristics of the response of type III cells. A high polymorphism of the elementary granules and their variable density, as well as reduced granule numbers, with a light surrounding zone osmiophilic membrane in cell type IV in the animals under continuous illumination indicate an active response of these cells to stress. Progressive aactvites were also observed in orbiting neuroglial cells correlating with the response of descnbed neuroglandular cells.

On the other hand, ultrastructural charactenstics of the type I neurosecretory cells in darkmaintained animals oppose to those under continuous light. When comapared with cell organells of the control, certain indications of a declining activity are observed. The type II cells are charctenzed by the occurrenceof cisternae, reduced number of neurosecretory granules, and intermitochondrial genesis of polymorphous lamelar bodies. When compared with the characteristics of the neurosecretory cells of he control, as well as those of cells in animals exposed to continuous light, a question arises as to whether they undergo the stage of declining activity? Their cytodynamics confirm this staatement. On the basis of the characteristics of type II cell organelles, one may conclude that they reflect cell declining synthesis and liberation of neurosecretion. In the type III cells, the occurrence of the lamelar structures is the most striking characteristic while vacuolaltion of the mitochondrial cristae in the cell type IV. When these two cell types are compared with those of the control, it may be concluded that they are characterized by a declining activity when compared either with synthesis or mobilization processes, i. e. neurosecretory granule secretion. The neuroglial cells of dark – affected animals are characterized by an increased number of dark bodies, higher motochondrial density, and well developed endoplasmic reticulum. Also, the occurrence of young, incompletely differentiated cells possibly corresponds to cells – restoration sources of neuroglandular cells. Comparing the dynamics of these cells with those of neuroglial onnes, a correlating response in imposed. Changes in the neurosecretory neurons of dark – influenced animals are depressive in character.

Mastocytes as a distinct cell type of neuroglandular nature and their occurrence in Lumbricida brain are worth to be noted due to the physiological characteristics of these cells belonging to the intercellular tissue. Such cell type was described for the first time in stress – exposed Lumbricus rubellus. According to our results we suppose that mastocytes, as separate cells, accompany brain structural

elemets of neuroglandular nature. From the point of stress – analysis a question might be defined: could each of the studied stress – gebnerated factors produce an effect equal to a joint one? O the basis of the obtained results we suppose that they are a consequence of a joint action of all the stress – generated factors. Another question as to whether we could foreseen certain other functional trais of neuroglandular cells using our investigations and the obtaied results also arises. Since the aim of our investigations did not encompass a systematic annalysis of reactive changes in other portions of the Lumbricida nervous system, the obtained brain results undoubtedly point to the adaptation syndrome theory. Accordingly, stress situations primarily mobilize adaptation, i. e. the aggression – resistance mechanisms. All the analyzed annimals experieced chronic – stress siluations concurrent with experimentally channged osmoregulation owing to increased humidity and increased temperature conditions. Adaptational mechannismis in the earthworm as a response to altered environmental conditions in the experiment comprised described reactive changes in the brain neurosecretory cells. On the basis of these data we suppose that these cells are sites of active principle secretion, i. e. neurohormones playing a crucial role in the adaptation phenomenon to the altered envirnomental conditions.