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ULTRASTRUCTURAL EFFECTS OF NICOTINE ADMINISTRATION ON SYMPATHETIC NERVOUS SYSTEM OF ADRENAL GLAND MEDULLA IN PATS: A OUALITATIVE STUDY BY ELECTRON MICROSCOPY

SIÇANLARDA NİKOTİN UYGULANMASININ SÜRRENAL BEZ MEDULLASININ SEMPATİK SİNİR SİSTEMİ ÜZERİNE OLAN ETKİLERİ: ELEKTRON MİKROSKOPLA GERÇEKLEŞTİRİLEN KALİTATİF BİR ÇALIŞMA

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Key vvords: adrenal medulla, sympathetic nervous system, electron microscopy, nicotine, rat.
Anahtar sözcükler: sürrenal medulla, sempatik sinir sistemi, elektron mikroskobi, nikotin, sıçan.

SUMMARY

Morphology of sympathetic nervous system of adrenal medulla was observed in VVistar rats injected with subcutaneous nicotine. They were *injected subcutaneously with low dose (2 mg/kg/day) or high dose (4 mg/kg/day) nicotine for seven weeks. Morphological changes in the medulla of adrenal glands vvere investigated in detail using electron microscopy. Uitrastructurally, we found that the appearance of sinusoids and endothelial cells containing epinephrine (E) and norepineprine (NE) granules vvere normal. İn both low and high dose nicotine treatment groups, the granules vvithin cells secreting E and NE were dense. Furthermore, in addition to an enlargment in the sinusoids of the adrenal medulla, depositions in the nuclei and an excessive vacuolisation due to increased secretion especialiy in NE secreting cells vvere noted. There was also hypertrophy of the vvalls of endothelial cells around sinusoids in animals treated vvith high-dose nicotine. We conclude that nicotine has a dose-dependent effect causing obvious morphological changes on the sympathetic nervous system of adrenal medulla in rats, suggesting its hypertensive effect.*

ÖZET

Subkütan nikotin enjeksiyonu uygulanan VVistar cinsi sıçanlarda sürrenal medulla sempatik sinir sistemi morfolojisi tetkik edilmiştir. Hayvanlara 7 hafta süreyle düşük dozda (2 mg/kg/gün) ve yüksek dozda (4 mg/kg/gün) nikotin subkütan olarak uygulanmıştır. Sürrenal bezlerin medullasındaki morfolojik değişiklikler elektron mikroskop kullanılarak ayrıntılı bir şekilde değerlendirilmiştir. Ultrastrüktürel olarak, epinefrin (E) ve norepinefrin (NE) içeren hücrelere ait sinüzoidlerin ve endotel hücrelerinin normal görünümde olduğu saptanmıştır. Hem düşük ve hem de yüksek doz nikotin uygulanan tedavi gruplarında, E ve NE salgılayan hücreler içinde yoğun granülasyon görülmüştür. Ayrıca, sürrenal medulla sinüzoidlehndeki genişlemeye ilave olarak, özellikle NE salgılayan hücrelerdeki sekresyon artışı nedeniyle ortaya çıkan aşırı bir vakuolizasyon ve nukleus içi depolanmalar dikkati çekmiştir. Yüksek doz nikotin uygulanan hayvanlarda sinüzoidlerin çevrelerinde yer alan endotel hücrelerinin duvarlarında bir hipertrofi mevcudiyeti saptanmıştır. Bu bulgularla,

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sıçanlarda nikotinin sürrenal medulla sempatik sinir sistemi üzerinde doza-bağımh olan ve antihipertansif bir etki gösterdiğini düşündüren belirgin morfolojik değişikliklerin geliştiği sonucuna varılmıştır.

INTRODUCTION

Adrenal giands consist of cortex and medulla which are different in terms of consîructional, functional and embryologica! developmenî. Adrenal medulla develops from crista neuralis and it is originally an ectodermal germ layer (1). Parencyma! cells of adrenal meduila which form anastomatic cords or groups are large and polygonal (1-5). Two different îypes of cells which secrete norepinephrine (NE) and epinephrine (E) in rats have been described (6). The cells secreting NE have peripheral location while those secreting E are central (7). Ultrastructurally, chromaffin cells contain granules surrounded by a membrane with 100-300 nm (4). NE cells have dense cores which were separated from the surrounding membrane by an eiectron lucent nimbus like space. The granuies of E cells are, hovvever, less dense and locked v v i t h l n the large elecîron iucent spaces (6). There are collagen fibriis and fibroblasts In the stroma of the medulla, and rareiy macrophages (6, 8). Preganglionic sympathetic cords similar to adrenal medulla come with the he!p of nervus splancnicus major and plexus coeliacus, and there is no parasyrnpathetic innervation. in comparison with their volıimes, interestingiy, adrenal giands have more autonomous innervation than the other organs (1, 7). Bunches of nerve fibres consist of fibres with or without myeiin. On adrenal medulla there are sympathetic ganglion cells lying singly or in small groups and dense nerve networks (2, 5). Nerve fibriis are ended in parenchymal cells of adrenal medulla, which are accepted as modified postganglionic neurons. The transmitter substance between the ending of nerve cells and secreting cells is acetylcholine (9). In adrenal medulla, there are nicotinic, muscarinic and dopaminergic receptors in E, NE and dopamine (DA) cells (10, 11}. The amount of E secreîed from adrenal medulla is about 80% (4).

Nicotine is a toxic substance which first accumulates in autonomous gangiions depending on its dosage. These ganglions are then blocked if exposure continues (12). Nicotine stimulates the secretions of catecholamines from sympathetic nerve endings and medulla of adrenal giands being effective at the peripheral nicotinic cholinergic receptors. İt can quickly pass blood-brain barrier and has a strong stimulant effect on central nervous system (13). The effects of nicotine on the nicotinic receptors at the centrai sympathetic centers that coordinate the activity of adrenal medulla and peripheral sympathetic nerves have been investigated previously (13). It has a dose dependent effect on adrenal medulla, where sympathetic innervation is rich, giving rise to catecholamine secretion (14). Some physiological and behavioural effects of nicotine are increased by adrenaiectomy, but decreased by acute and chronic administratiön of corticosteroids (15). Many investigators have focused on toxic effects of nicotine on caîecholamine secretion, but its possibie hazardous effect is not well documented regarding morphoiogica! changes In the adrenal medulla (13, 16-18). The effects of central sympathetic nervous system, peripheral sympathetic nerves and adrenal medulla on the deveiopment of genetic hypertension In the humans and experimental hypertension of the animals have been studied (1, 9, 19-21). We reported iight microscopicai results of nicotine administratiön In the first part of the study (22). In the present paper, we report ultrastructural alterations in sympathetic nervous system of adrenal medulla after nicotine adminstration in rats.

MATERLAL AND METHOD

In this study, laboratory bred, sexually mature, 8-10-week-old Wistar rats, weighing 110-140 g, were used and were kept under standard housing conditions with free access to water and standard pellet diet. A total of 40 animals were firstly segregated into two groups: uninjected control (n=8) and experimental group (n=32). Thereafter, the nicotine-injected experimenta! group was divided into two subgroups. In the first subgroup (n=16) 2 mg/kg/day, and In the second (n=16), 4mg/kg/day nicotine were injected subcutaneously for seven weeks. Ali experimental procedures were performed in accordance with universal ethical principles. In the experirnent solution, nicotine sulphate (moiecular weight is 442.5), supplied from Sigma Chemical Co. (St. Louis, MO), has been used. At the end of the study, the rats have been decapited and the parts taken from the adrenal giands of the rats both In the control and experimental group were processed with 1% osmium tetroxide postfixation after primary 2.5% glutaraldehyde fixation. After routine fixation processes they were dug into Epon 812, and semithin seriai sections were taken from epon blocks in Reichert Ultramicrotome. Appropriate areas were selected for eiectron microscopy and ultrathin sections were stained with uranyl acetate, lead citrate and examined by Zeiss EM 9 transmission eiectron microscope (Zeiss, Germany) for ultrastructural morphology.

RESULTS

In the control group, the celis which contain E and NE granules In the rat adrenal medulla were evaluated separately. The cells secreting E and NE were normal in appearance and these resuits were in harmony with the

observations in the literatüre (Figure 1). The groups, in which 2mg/kg/day and 4 mg/kg/day nicoîine were given respectiveiy, showed an increase in density of granules within both the E and NE cells (Figure 2 and 3). Especially In the second group (4mg/kg/day nicotine) excessive secretion vvithin E and NE secreting cells was noted. Referred to the increase in secretion, depositions In the nuclei of the cells, especially in those secreting NE, were seen occasionally (Figure 2). An excessive vacuolisaîion due to increased secretion In the NE and E cells was evident (Figure 4 and 5). An eniargement In the sinusoids of the adrenal medulla due to increased secretion was noted (Figure 5). Granule density in NE and E cells of sinusoids was high (Figure 5). Around the sinusoids, endothelial cells were noted with increase in wall thickness and intracytoplasmic granules (Figure 6).

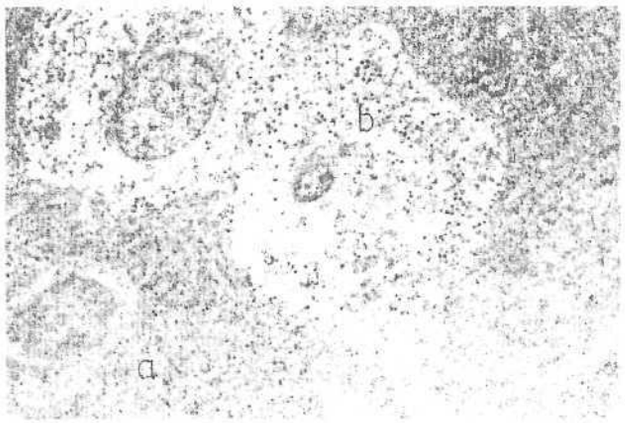

Figure 1. NE (a) and E (b) secreting cells In the adrenai medulla of rats in controi group (x1750).

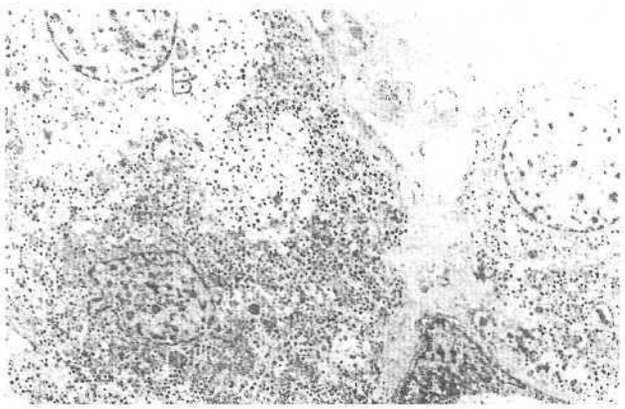

Figure 2. NE (A) and E (B) secreting celis In the adrenal medulla of rats in low-dose nicotine treatment group (x1750).

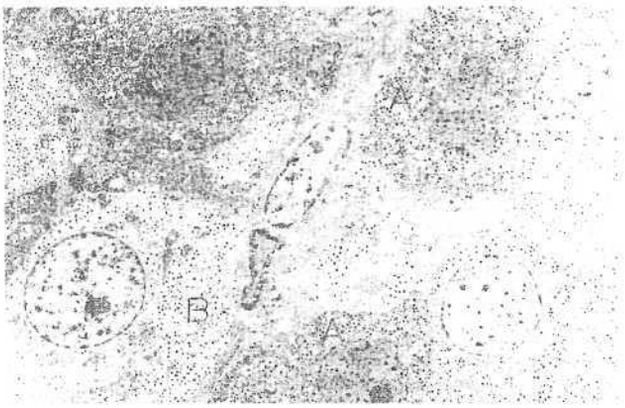

Figure 3. Excessive secretion in NE (A) and L (B) secreting cells In the adrenal medulla of rate in high-dose nicotine treatment (x1750).

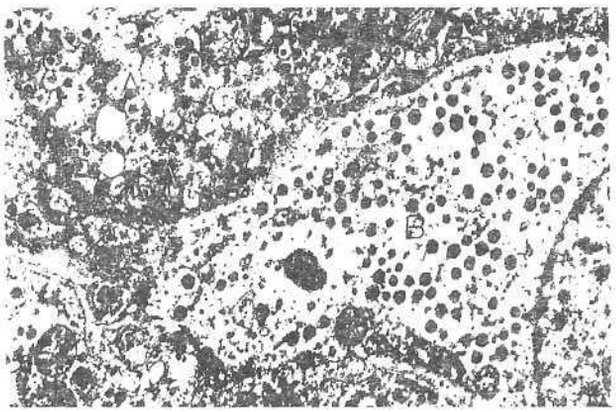

Figure 4. The adrenal medulla of rats in high-dose nicotine treatment group. Increased vacuoiisation and empty granules v v i t h i n the NE (A) secreting cells on left side. Increased granules In the cells secreting E (B) on right side (x7200).

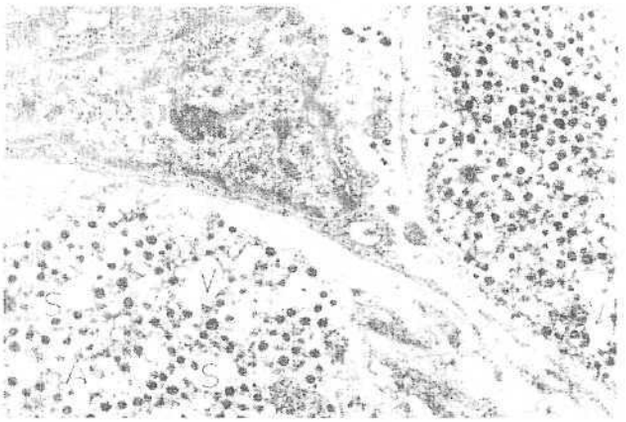

Figure 5. High-dose nicotine treatment group: increased secretion, vacuolisalion (V) and an electron lucent spaces (S) between the secretion in NE (A) secreting cells and the surrounding membrane on the left side **(bottom),** and nucleus (N) In the stroma of the fibroblast left side (top) **(x7200).**

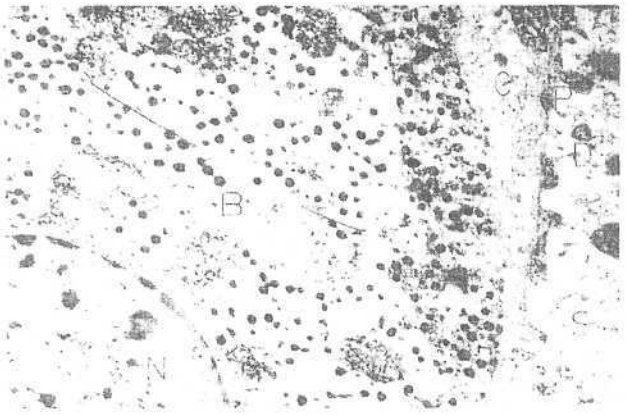

Figure 6. Low-dose nicotine treatment groıip. E secreting celi (B) and its nucleus (N) on the left side, and endotheüa! celi (C) and granules as weil as sinusoid (S) on the ride side. Note that hypertrophy of the walls (P) of endothelial cells and deposits (D) of these cells w i t h I n the sinusoid (x7200).

DISCUSSION

In the adrenal meduila, the activity of cells defined as modified postganglionic neurons is under the neural control (3). The transmitter substance betvveen the nerve cells and the chromaffin ceils in adrenal meduila is acetylcholine (9). Similar to acetylcholine, nicotine has an effect on the nicotinic receptors (9). There are nicotinic, muscarinic and dopaminergic receptors in chromaffin ceils (10,11), Nicotinic receptors intervene the secretion of both E and NE (9-11,17). The effects of the nicotine on the peripheral sympathetic system have also been knovvn (12,17). Nicotine adminisîration intracerebroventricularly in low or high dosage to the rats increases the secretion of E and NE In the plasma (13). Nicotinic receptors also help nicotine to intervene on the upper sympathetic centers nicotinic receptors. In this study, we showed that nicotine is responsible for hypertrophy of cells within adrenal meduila indicating an increase in secretion by E and NE ceils. Nicotine shows this effect of by different mechanisms, particularly arousing the secretion of adrenocorticotrophic hormones from pituitary glands, centrai sympathetic centers and periphera! sympathetic nerves (13, 17, 23). A close relationship betvveen the increased secretion of catecholamine and hypertension has been reported by several studies (11, 20, 21, 24).

Experimental studies as well as clinical trials on hypertensive patients showed that catecholamine levels at the ends of sympathetic nerves within adrenal meduila are elevated (20, 21). The molecular mechanisms of hypertension deveiopment in centrai sympathetic nerves have also been investigated (11, 19-21, 24-26). Recently, Yokotani et al. (26) suggested that alpha3beta4 nicotinic receptors were involved In the release of catecholamines from the rat adrenal gland. Our previous study using light microscopy suggests that hypertrophy of chromaffin cells v v i t h I n the adrenal meduila in experimental group is resulted from an excessive stimulation of these cells (27). İn rats, long-îerm parenteral nicotine administration caused hypertrophy of the cells secreting renin in juxtaglomerular apparatus and thus ieading excessive renin secretion (22). On the other hand, it was noted that nicotine and its metabolites caused direct and specific inhibition of aldosteron synthesis resulting in cardiovascular pathologies by activation of renin-angiotensin system (28).

Experimental studies indicated that, in rats with hypertension, an increase in E levels, svveels on the sinusoid endotheis, and accumulation of granules within E cells occur (24). The authors have stated that these findings are morphoiogical evidences indicating a significant relationship betvveen hypertension and E secretion (7). Our study shows that similar morphoiogical changes on adrenal meduila are also suggestive for hypertensive effect of nicotine. Based on the literatüre and our results, we suggest that nicotine has a hypertensive effect, although further experirnental and clinical studies are required for final decision.

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