Acta Veterinaria (Beograd), Vol. 49. No. 2-3, 89-96, 1999.

UDK 619:612.017.2

CENTRAL AND PERIPHERAL CATECHOLAMINE STORES IN SPONTANEOUSLY HYPERTENSIVE RATS UNDER IMMOBILIZATION STRESS

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(Received, 10. March 1999)

The effects of increasing duration of immobilization stress (from 30 min to 240 min) on catecholamine stores in some central (hypothalamus, corpus striatum) and peripheral tissues (suprarenal glands, heart auricles) in spontaneously hypertensive rats were investigated. Sufficiently long immobilization induced significant depletion of both central and peripehral catecholamine stores. The greatest depletion occurred in the suprarenal gland(adrenaline) and in the hypothalamus (noradrenaline and dopamine). The corpus striatum (dopamine) and the heart auricles (noradrenaline) were the most resistant to immobilization stress. These data indicate that catecholamine depletion in all investigated structures in spontaneously hypertensive rats depended on the duration of immobilization. It was concluded that after immobilization for more than 30 min the release of catecholamines was stronger that the processes of synthesis and reuptake, thus leading to progressive depletion of catecholamine stores, particularly when imobilization lasted up to 240 min. The present results indicate a difference in the effects of immobilization stress on catecholamine stores between some brain regions and peripheral tissues in spontaneously hypertensive rats.

Key words: immobilization, catecholamine, tissues

INTRODUCTION

It was found previously that sufficiently long immobilization produced significant depletion of both central and peripehral catecholamine stores in normotensive Wistar rats (Nikolić et al., 1993). Spontaneously hypertensive rats (SHR) have been used as a model for studying processes occuring in the development of hypertension (Nishibayashi et al., 1995). Catecholamine stores under resting conditions have already been studied (Dronjak et al., 1996). It was of interest to investigate some central and peripheral catecholamine stores after immobilization stress in SHP.

MATERIALS AND METHODS

The experiments were carried out on male spontaneously hypertensive rats weighing 300-330 g. They were bred and kept under ordinary laboratory conditions with water and food ad libitum. The rats were immobilized by fixing them to a board. All four limbs of the animals were fixed to the board by sticky tape. The head was also fixed by a metal loop over the neck area of the animal, thus limiting the motion of the head (Kvetnansky and Mikulaj, 1970; Kvetnansky et al., 1978). Immobilization lasted for 30, 60, 90, 120 and 240 min, respectively, when the animals were decapitated. Corpus striatum, hypothalamus, heart auricles and adrenals were dissected out immediately after decapitation and immersed in cold (4^0C) 0.1 $NCL0_4$ (0.3 μg of tissue per 30 μg of 0.1 N $HCLO_4$). The tissue was then homogenized (10.000 rpm per min for 15 min). The supernatant (30 μl) was used for analysis.

Catecholamines in tissues were determined using a modified method of Da Prada and Zurcher (1976), Weise and Kopin (1976) and Peuler and Johnson (1977). The principle of the method was to convert the catecholamines into the corresponding 0-methyl derivatives using purified catechol-0-methyl-transferase (COMT) in the presence of S-adensosyl-1-(^{3}H-methyl)-methionine. The O-methyl derivatives were then extracted and oxidized to ^{3}H-vanillin. The activity of this substance was measured using a liquid scintillation counter (Packard).

The statistical significance of differences between the results was evaluated by Student's t-test.

The following substances were used: S-adenosyl-1-(^{3}H-methyl)-methionine (Amersham U. K.) and EGTA. COMT was prepared according to the method of Axelrod and Tomchick (1958).

RESULTS

Both noradrenaline and dopamine are present in the hypothalamus, but only dopamine in corpus striatum. The results presented in Figure 1., Figure 2 and Fig. 3. show that immobilization for 30 min did not produce any significant change in catecholamine levels in these central structures. Even immobilization for 60 min did not produce either a significant depletion of noradrenaline and dopamine in the hypothalamus, or of dopamine in corpus striatum. Immobilization stress lasting from 90 to 240 min resulted in either significant or highly significant decreases in the catecholamine stores in both hypothalamus and corpus striatum compared with initial values.

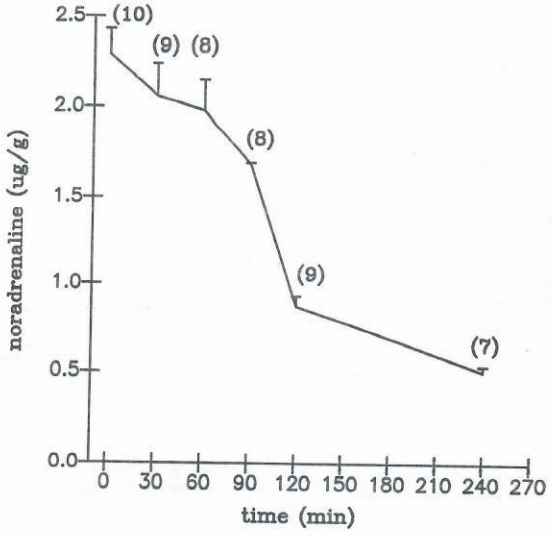

Figure 1. The amount of noradrenaline (in μg/g of fresh tissue) in the hypothalamus of SHR after periods of immobilization ranging from 30 to 240 minutes. The number of animals is shown in parentheses. Abscissa: time indicating the duration of immobilization. Ordinate: amount of noradrenaline (μg/g tissue).

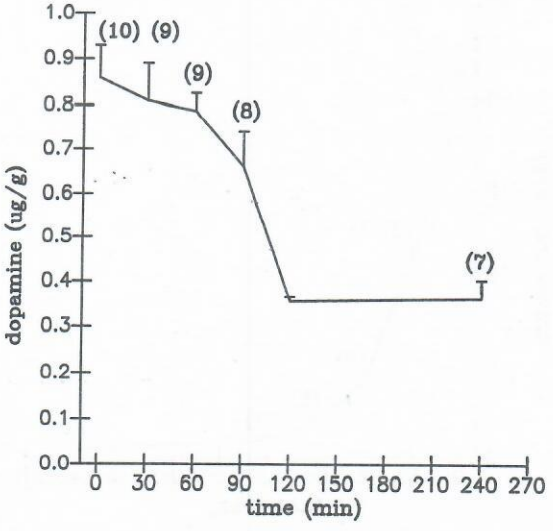

Figure 2. The amount of dopamine (in μg/g of fresh tissue) in the hypothalamus of SHR after periods of immobilization ranging from 30 to 240 minutes. The number of animals is shown in parentheses. Abscissa: time indicating the duration of immobilization. Ordinate: amount of noradrenaline (μg/g tissue).

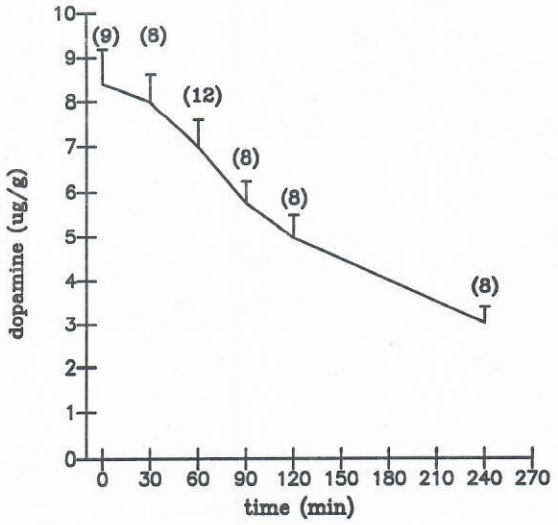

Figure 3. The amount of dopamine (in μg/g of fresh tissue) in the corpus striatum of SHR after periods of immobilization ranging from 30 to 240 minutes. The number of animals is shown in parentheses. Abscissa: time indicating the duration of immobilization. Ordinate: amount of noradrenaline (μg/g tissue).

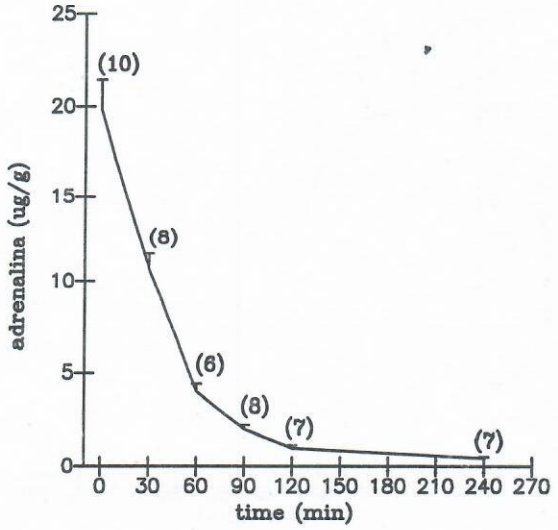

Figure 4. The amount of adrenaline (in μg/g of fresh tissue) in the adrenal glands of SHR after periods of immobilization ranging fromm 30 to 240 minutes. The number of animals is shown in parentheses. Abscissa: time indicating the duration of immobilization. Ordinate: amount of noradrenaline (μg/g tissue).

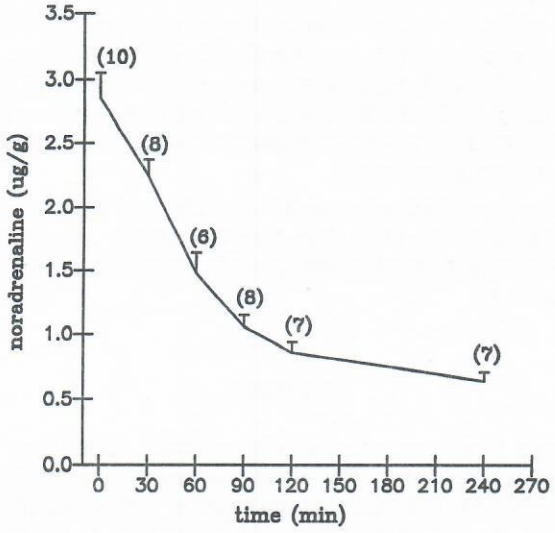

Figure 5. The amount of noradrenaline (in μg/g of fresh tissue) in the adrenal glands of SHR after periods of immobilization ranging from 30 to 240 minutes. The number of animals is shown in parentheses. Abscissa: time indicating the duration of immobilization. Ordinate: amount of noradrenaline (μg/g tissue).

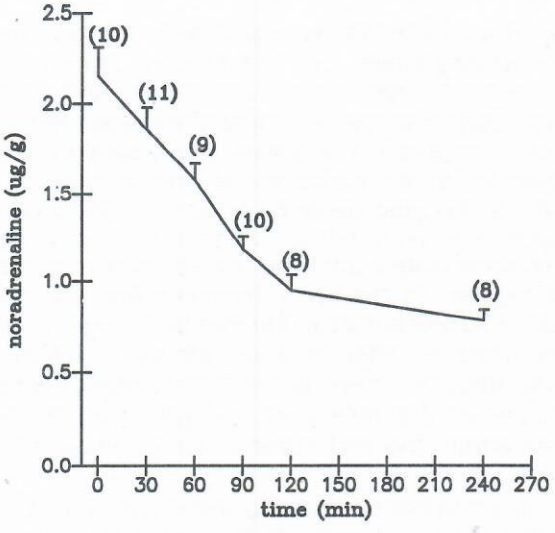

Figure 6. The amount of noradrenaline (in μg/g of fresh tissue) in the heart auricles of SHR after periods of immobilization ranging from 30 to 240 minutes. The number of animals is shown in parentheses. Abscissa: time indicating the duration of immobilization. Ordinate: amount of noradrenaline (μg/g tissue).

It is evident from the results presented in Figure 4. and Figure 5 that even a relatively short period of immobilization stress (30 min) produced a highly significant decrease in the amount of adrenaline in the suprarenal gland. The percentage decrease of adrenaline was greater than that of noradrenaline during the same period of immobilization. All periods of immobilization stress from 60 to 240 min produced further drastic depletions of both adrenaline and noradrenaline in the suprarenals.

A shortlasting immobilization of 30 min did not produce any significant change in noradrenaline level in the heart auricles. However, a significant decrease in noradrenaline level in the auricles was observed after 60 min of imobilization. (Figure 6).

DISCUSSION

Immobilization stress produces a significant depletion of central and peripheral catecholamine stores. The strongest and quickest depletion occured in adrenaline stores in the suprarenal gland. Immobilization for 30 min decreased the level of adrenaline in the suprarenal gland to 45% of the control values. Depletion of noradrenaline stores in the adrenals was less pronounced. Even the longest period of immobilization depleted noradrenaline stores down to 25%. During immobilization, noradrenalines mainly released from sympathetic terminals, with a minor contribution from the adrenal glands. In contrast, virtually all of the adrenaline release is from adrenomedullary tissue (Kvetnansky et al, 1979).

Immobilization for 30 min did not significantly change noradrenaline and dopamine in the hypothalamus, but the longest period of immobilization decreased markedly the level of noradrenaline and dopamine in the hypothalamus. If immobilization lasted longer, the release of noradrenaline and dopamine resulted in progressive depletion. Immediately after acute stress, a significant reduction in noradrenaline levels and a marked increases in 3-methoxy- 4- hydroxyphenylethyleneglycol sulfate (MHPG) levels in the hypothalamus, hippocampus, cerebral cortex and locus coeruleus were observed. In contrast, immediately after the last stressor of a chronic or chronic intermittent stress regime, no change in noradrenaline concentration was observed, while levels of MHPG-sulfate in the four brain regions showed a smaller increase than that observed after an acute stressor (Hellriegel and D'Mello, 1997). The hypothalamus is known to be an important center for regulation of catecholamines. Electrical stimulation of the hypothalamus resulted in specific changes in plasma adrenaline and noradrenaline concentrations (Bereiter et al., 1986).

The most resistant to immobilizations stress were the stores of dopamine in Corpus striatum and noradrenaline in heart auricles. Even the longest immobilization depleted the NA stores in the heart auricles only to 56% in comparison to the control value. After 90, 120 and 240 min of immobilization, dopamine stores in Corpus striatum were decreased.

The data obtained in the present experiments indicate that the degree of catecholamine depletion in central and peripheral tissues depends on the duration of immobilization. Still, even the same stressor can produce changes in the catecholamine levels to various degrees in various tissues.

Immobilization stress increases the turnover of endogenous catecholamines which are matched by increases in catecholamine synthesis. Our experiments indicate that after immobilization lasting for more than 30 min the release of catecholamines from both central and peripheral tissues is stronger than the processes of synthesis and reuptake, thus producing a progressive depletion of catecholamine stores.

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CENTRALNI I PERIFERNI DEPOI KATEHOLAMINA KOD SPONTANO HIPERTENZIVNIH PACOVA POD DEJSTVOM IMOBILIZACIJSKOG STRESA

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SADRŽAJ

Ispitivano je dejstvo imobilizacijskog stresa u različitom vremenskom periodu (30 do 240 minuta) na depoe kateholamina u nekim centralnim (hipotalamus, corpus striatum) i perifernim tkivima (nadbubrežne žlezde, srčane pretkomore) kod spontano hipertenzivnih pacova. Dovoljno duga imobilizacija prouzrukuje značajno smanjenje kako centralnih tako i perifernih deopoa kateholamina. Najjače smanjenje nastaje u nadbubrežnim žlezdama (adrenalin) i hipotalamusu (noradrenalin). Corpus striatum (dopamin) i srčane pretkomore (noradrenalin) su najotpornije na imobilizacijski stres. Naši podaci ukazuju da smanjenje kateholamina u svim ispitivanim strukturama kod spontano hipertenzivnih pacova zavisi od dužine trajanja imobilizacije. Na osnovu izloženih podataka možemo zaključiti da posle imobilizacije koja traje duže od 30 minuta oslobađanje kateholamina iz centralnih i perifernih deopa je jače nego procesi sinteze i preuzimanja, što dovodi do procesa osiromašenja kateholaminskih deopa. Rezultati ukazuju na postojanje razlike u dejstvu imobilizacijskog stresa na deope kateholamina između nekih moždanih regiona i perifernih tkiva kod spontano hipertenzivnih pacova.