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Effects of stress on catecholamine stores in central and peripheral tissues of long-term socially isolated rats

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Abstract

Both the peripheral sympatho-adrenomedullary and central catecholaminergic systems are activated by various psycho-social and physical stressors. Catecholamine stores in the hypothalamus, hippocampus, adrenal glands, and heart auricles of long-term socially isolated (21 days) and control 3-month-old male Wistar rats, as well as their response to immobilization of all 4 limbs and head fixed for 2 h and cold stress (4ºC, 2 h), were studied. A simultaneous single isotope radioenzymatic assay based on the conversion of catecholamines to the corresponding O-methylated derivatives by catechol-O-methyltransferase in the presence of S-adenosyl-l-(^{3}H-methyl)-methionine was used. The O-methylated derivatives were oxidized to ^{3}H-vanilline and the radioactivity measured. Social isolation produced depletion of hypothalamic norepinephrine (about 18%) and hippocampal dopamine (about 20%) stores and no changes in peripheral tissues. Immobilization decreased catecholamine stores (approximately 39%) in central and peripheral tissues of control animals. However, in socially isolated rats, these reductions were observed only in the hippocampus and peripheral tissues. Cold did not affect hypothalamic catecholamine stores but reduced hippocampal dopamine (about 20%) as well as norepinephrine stores in peripheral tissues both in control and socially isolated rats, while epinephrine levels were unchanged. Thus, immobilization was more efficient in reducing catecholamine stores in control and chronically isolated rats compared to cold stress. The differences in rearing conditions appear to influence the response of adult animals to additional stress. In addition, the influence of previous exposure to a stressor on catecholaminergic activity in the brainstem depends on both the particular catecholaminergic area studied and the properties of additional acute stress. Therefore, the sensitivity of the catecholaminergic system to habituation appears to be tissue-specific.

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Key words
- Cold stress
- Immobilization stress
- Catecholamines
- Hypothalamus
- Hippocampus
- Adrenal glands

Introduction

Stress disturbs homeostasis and may induce various disorders. Both the peripheral sympatho-adrenomedullary and central monoaminergic systems are activated by various psycho-social and physical stressors. Limbic circuits connecting, e.g., the hippocampus, amygdala and prefrontal cortex are sensitive to stressors such as restraint, fear or exposure to a novel environment. In contrast, physiological threats such as exposure to ether result in the activation of efferent visceral pathways that are directly relayed to the paraventricular nucleus of the hypothalamus (1). Activation of limbic and hypothalamic brain structures is a major component of the stress reaction that integrates neuroendocrine and emotional components and thus determines the magnitude and duration of the hormonal and neural stress response. Recent reports have suggested that different types of stress may sometimes produce qualitatively different patterns of effects on both behavior and physiology. Using an *in vivo* microdialysis method, Tanaka et al. (2,3) reported that the same immobilization stress led to a different time course of changes in norepinephrine (NE) levels in the amygdala when compared to the hypothalamus, suggesting regional differences in release due to functional differences in the stress response. Shibasaki et al. (4) showed that the response pattern of NE release to repeated stress in the hypothalamus was also dependent on the type of stressor employed, since restraint stress, but not tail-pinching, promotes desensitization of hypothalamic NE neurons. Animals exposed to prior stress exhibit an enhanced, reduced or equivalent hypothalamic-pituitary-adrenal response to a subsequent acute stressor (5-7). Social isolation produced by individual housing affects brain monoamine and adrenal gland functions in experimental animals (8-11). Social isolation and acute environmental changes are risk factors in human depression, and represent a lack of the social stimuli necessary to modulate adaptive responses to new situations (12). The differences in animal rearing conditions appear to influence the responses to acute stress. Almost all reported studies have examined the effects of isolation during the weaning period. However, Miura et al. (13) suggested that animals suffering isolation in adulthood also show behavioral changes, indicating that isolation can alter the central nervous system activity of adult animals previously reared in a group.

We investigated the influence of long-term social isolation on catecholamine stores in central and peripheral tissues in response to two different types of additional stressors, i.e., immobilization and cold.

Material and Methods

Three-month-old male Wistar rats weighing 300-340 g maintained under standard laboratory conditions with water and food *ad libitum* were used. Before being subjected to stress the animals were housed in groups of four individuals. The animals were divided into two groups, a control group consisting of four animals per cage and a group of animals that were housed individually for the 21-day study period. Both groups were exposed to short-term (2 h) immobilization or cold stress. The animals were treated as humanely as possible according to the recommendations of the "Vinča" Institute which are based on the Guide for Care and Use of Laboratory Animals of the National Institutes of Health, Bethesda, MD, USA.

Rats were immobilized in the prone position with all four limbs fixed to the board with adhesive tape. The head was also fixed with a metal loop over the neck area, with a consequent limitation of head motion (14). The animals exposed to cold stress were carefully transferred to a cold chamber at 4ºC. After either immobilization or exposure to cold, both lasting 2 h, the rats were

decapitated and the hippocampi, hypothalami, heart auricles, and adrenal glands were quickly dissected and immersed in cold 0.1 N $HClO_4$ (0.3 µg tissue per 30 µL 0.1 N $HClO_4$ at 4ºC). The tissues were then homogenized in a motor-driven homogenizer and centrifuged at 10,000 rpm for 20 min at 4ºC in a K24 Janetzky centrifuge (Berlin, Germany). The resulting supernatant solutions (30-µL aliquots) were stored at -70ºC and later used for the analyses.

Catecholamines in the tissues were determined using the single isotope radioenzymatic assay of Peuler and Johnson (15) based on the conversion of catecholamines to the corresponding O-methylated derivatives by purified catechol-O-methyl-transferase in the presence of S-adenosyl-l-(^{3}H-methyl)-methionine. The O-methylated derivatives were oxidized to ^{3}H-vanilline. Radioactivity was measured with a toluene-based scintillation liquid and with an LKB-Wallac model 1219 scintillation counter (Stockholm, Sweden) at 40% efficiency for tritium. The range of measurement is Window 1 5-320, sensitivity is 20 CPM and interassay is less than 10%.

Data were analyzed statistically by two-way ANOVA, with the level of significance set at $P < 0.05$.

Results

The results presented in Table 1 show that when compared with naive control, long-term isolation produced a significant depletion of only NE stores (about 18%, $P < 0.05$) in the hypothalamus, while in the hippocampus a significant decrease was observed only in dopamine (DA) content (about 20%). Social isolation produced no significant changes in epinephrine (E) or NE levels in adrenal glands, or in heart auricle NE levels.

Immobilization stress significantly decreased both NE ($P < 0.001$) and DA ($P < 0.01$) stores in the hypothalamus (about 36%) and hippocampus (approximately 39%) of the controls compared with zero point (0'), as well as in the hippocampus of long-term isolated rats (approximately 20%; $P < 0.05$) compared with 0'. However, immobiliza-

Table 1. Changes in catecholamine stores due to social isolation.

	Control			Isolation		
	0'	Immo	Cold	0'	Immo	Cold
Hypothalamus						
NE	1.94 ± 0.22	1.23 ± 0.14^{+++}	1.91 ± 0.22	1.60 ± 0.17*	1.58 ± 0.17*	1.63 ± 0.2*
DA	0.78 ± 0.09	0.50 ± 0.06^{++}	0.75 ± 0.09	0.75 ± 0.09	0.72 ± 0.09*	0.71 ± 0.093
Hippocampus						
NE	1.01 ± 0.12	0.63 ± 0.08^{+++}	0.93 ± 0.13	0.98 ± 0.11	0.77 ± 0.09*$^{+}$	0.91 ± 0.12
DA	1.11 ± 0.13	0.66 ± 0.08^{+++}	0.88 ± 0.1^{+}	0.89 ± 0.1*	0.72 ± 0.09^{+}	0.75 ± 0.095*$^{+}$
Adrenal glands						
NE	2.35 ± 0.25	1.09 ± 0.13^{+++}	1.87 ± 0.2^{+}	2.39 ± 0.26	1.26 ± 0.15^{+++}	1.97 ± 0.22
E	20.74 ± 2.22	5.63 ± 0.7^{+++}	19.52 ± 2.1^{+}	21.01 ± 2.3	5.95 ± 0.7^{+++}	19.76 ± 2.3
Heart auricles						
NE	2.01 ± 0.2	1.45 ± 0.17^{+++}	1.72 ± 0.19^{++}	1.95 ± 0.23	1.56 ± 0.17^{+++}	1.66 ± 0.18^{++}

Catecholamine stores were measured by the single isotope radioenzymatic method of Peuler and Johnson (15). The means and SEM for each of the two central and two peripheral tissues were calculated for each group of 6 animals. Social isolation was compared with naive control. Immobilization and cold stress were compared with their zero point. NE = norepinephrine; E = epinephrine; DA = dopamine; Immo = immobilization; cold stress = 0' to 2 h.

*$P < 0.05$ for 21-day social isolation *vs* naive control (two-way factorial ANOVA). $^{+}P < 0.05$; $^{++}P < 0.01$; $^{+++}P < 0.001$ for immobilization and cold stress compared to their zero point (two-way factorial ANOVA).

tion had no effect on the level of these catecholamines in the hypothalamus of animals previously submitted to long-term social isolation. Immobilization stress produced a highly significant decrease ($P < 0.001$) in the adrenal gland content of E (about 72%) and NE (about 50%) of both naive controls and rats previously exposed to long-term social isolation compared with 0'. Also, immobilization led to a decrease of heart auricle NE stores in both the control (about 28%) and long-term isolated rats (about 29%).

Cold stress did not reduce hypothalamic catecholamine stores in either control or long-term isolated rats compared with 0'. It also did not affect the hippocampal NE stores, while a statistically significant decrease ($P < 0.05$) of DA stores was observed in this brain region in the controls (about 21%) and long-term isolated rats (about 16%) compared with 0'. Likewise, cold stress induced a reduction ($P < 0.05$) of NE in the adrenal glands (about 20%) and in heart auricles (about 14%), but did not affect E stores in the adrenal glands of naive controls or the rats previously exposed to long-term isolation compared with 0'.

Discussion

Several studies have demonstrated that animals reared in isolation show some behavioral and neurochemical changes (16,17). The results of almost all earlier studies concerning the effects of social isolation were obtained by applying isolation at the beginning of the weaning period when the isolation conditions could influence the growth and development of the central nervous system. To eliminate this possibility, in the present study adult rats were subjected to 21-day isolation. The present data show that long-term social isolation led to a significant depletion of hypothalamic NE stores, without affecting hippocampal NE stores. The hypothalamus is the brain structure that influences all limbic structures and hypothalamic neurons are responsive to information arising in the internal and external environment. The hypothalamus controls the visceromotor responses by regulating the balance of sympathetic and parasympathetic outputs to the autonomic nervous system. The reduction of NE stores observed in the hypothalamus may indicate that neurons derived from the lateral tegmental system respond more powerfully to stress than those in the hippocampus derived from the locus ceruleus. The present results corroborate the finding that the basal hippocampal NE efflux does not differ between socially isolated and group-reared rats (11). The change in brain DA levels after acute and chronic exposure to stress may have different effects on this neurotransmitter. For example, in the prefrontal cortex, increased extracellular DA levels are observed after acute stress, but chronic stress reduces DA output (18,19). Our results show that immobilization significantly decreased DA, but not NE, content in the hypothalamus and hippocampus of control animals and significantly decreased the NE and DA content of the hippocampus of socially isolated animals. The results of Jedema et al. (20) suggest that the characteristics of the chronic stressor itself, as well as the pattern of exposure, play a role in the development of enhanced activity of the central NE neurons. The acute cold stress applied in our experiments did not reduce the hypothalamic catecholamine stores of either control or long-term isolated rats. This agrees with the results of Gilinskii et al. (21) who found that cold stress did not affect hypothalamic DA content. However, cold stress reduces the DA stores in the hippocampus of control and long-term isolated rats. Our results support the view that the brain categorizes stressors and activates neural response pathways that change according to each category. Dayas et al. (22) suggested that the brain recognizes at least two major categories of stressors referred to as physical and psychological. Our results showed that long-

term social isolation resulted in the reduction of DA stores in the hippocampus but not in the hypothalamus. Baffi and Palkovits (23) have reported similar results regarding emotional changes, with exposure to a stress situation increasing DA metabolite levels in the hippocampus. The results obtained in the present study showed that the influence of previous exposure to a stressor on catecholaminergic activity in the brainstem depends on the particular catecholaminergic area of the brainstem studied and on the properties of additional acute stress. Thus, the sensitivity of the catecholaminergic system to habituation appears to be region-specific.

Long-term social isolation produced no significant changes in the catecholamine levels of peripheral tissues, suggesting adaptation of the sympathetic adrenomedullary system to long-term isolation. It has been reported that exposure of animals to chronic intermittent stress results in a significant increase of catecholamine biosynthesis and storage capacity in peripheral tissues compared to unstressed controls (24,25). Animals exposed to repeated immobilization stress (41 days) showed permanently increased adrenal tyrosine hydroxylase mRNA levels, tyrosine hydroxylase activity and protein levels (26). Our results showed that additional immobilization stress produced a highly significant decrease in the amount of E and NE in the adrenal gland of long-term isolated rats. This could mean that these animals increasingly mobilized high stores of tissue catecholamines after exposure to an additional stressor. Cold stress induced only a reduction of NE stores in the adrenal glands and heart auricles of both naive control and long-term isolated rats. These results are in agreement with previous data obtained from our laboratory, that demonstrated an increased plasma level of NE upon exposure of rats to cold stress, while plasma E content remained unchanged (27).

The results of the present study showed that additional immobilization stress produced a greater reduction of catecholamine stores in all tissues examined in both control and chronically stressed rats compared to that observed after exposure of animals to a novel additional cold stress. Therefore, we conclude that the properties of the additional stressor play a role in the intensity of the response of peripheral and central noradrenergic neurons. In addition, the differences in rearing conditions of adult animals appear to influence their responses to additional stress.

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