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ORIGINAL ARTICLE

Activation of rat pituitary-adrenocortical and sympatho-adrenomedullary system in response to different stressors

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Abstract

OBJECTIVE: The effects of three different long-term (21 days) stresses: isolation (LTI), forced swimming (LTS) and isolation accompanied by forced swimming (LTI+LTS) on the level of plasma noradrenaline (NA), adrenaline (A), corticosterone (CORT) and adrenocorticotropic hormone (ACTH), both under basal conditions and in response to short-term immobilization and cold as heterotypic additional stressors, were compared.

METHODS: Plasma NA and A were assayed by the radioenzymatic method. Plasma CORT was measured using RIA kits. Plasma ACTH was determined by chemiluminescent method.

RESULTS: LTI produced a significant elevation of basal plasma CORT and ACTH, while basal plasma NA and A concentrations remained unchanged. Combination of long-term isolation and forced swimming, produced a significant elevation of basal plasma ACTH content only, while LTS did not influence the basal level of this hormone. When LTI rats were exposed to immobilization and cold, a significant elevation of plasma NA and A level was recorded. In LTS and LTI+LTS groups of rats exposed to immobilization or cold, increased plasma NA and A levels were observed, but this increase was lower in comparison with that found in LTI rats. No difference in plasma CORT content between the three long-term stressed groups of animals was observed, while plasma ACTH level was significantly more elevated in LTS and LTI+LTS than in LTI rats.

CONCLUSION: Based on these results, it may be concluded that LTI as a psychosocial stress represents a stronger stressor than LTS. Also, daily short-term (15 min, 21 days) swimming stress seems to attenuate the effect of long-term isolation on the activity of sympatho-adrenomedullary system.

Abbreviations

A	– adrenaline
NA	– noradrenaline
CORT	– corticosterone
ACTH	– adrenocorticotropic hormone
LTI	– long-term isolation
LTS	– long-term forced swimming
LTI+LTS	– long-term isolation accompanied by forced swimming

Introduction

The hypothalamo-pituitary-adrenocortical and sympatho-adrenomedullary systems interact complexly to maintain homeostasis upon exposure of an organism to different stressors. Applying a wide variety of stressors, several authors have clearly indicated that the pattern of neuroendocrine response is dependent on the stress stimulus applied [1,2]. Immobilization of experimental animals was shown to produce a rapid increase in secretion of noradrenaline (NA) and adrenaline (A) [3], while cold stress induced somewhat lower elevation of NA level comparing to immobilization, but did not affect A secretion [4]. We have observed previously, a potentiation of the sympatho-adrenaomedullary system activity in rats chronically exposed to cold or immobilization and additionally exposed to acute action of new stressors [5]. Chronic stress is considered to be one of the most important precipitating factors in cardiovascular and mental disorders. However, in recent years a large body of evidence has accumulated suggesting that physical exercise expressed positive effects on cardiovascular and immune system, as well as on the brain. It has been found that voluntary exercise increased neurogenesis in the dentate gyrus of the rat and mice hippocampus [6]. On the other hand, van Praag et al. [7] recorded a decrease in neurogenesis in rats or mice exposed to psychosocial stress, since social interactions are an important source of stress. Individual housing of rats, frequently termed "isolation stress", represents a very strong psychosocial stress. Physical stress involves both physical and emotional component, while psychosocial stress does not involve a physical component.

Taking into account the above studies, the aim of the present work was to investigate changes in the activity of pituitary-adrenocortical and sympatho-adrenomedullary system in adult rat males exposed to a long-term psychosocial (21 days isolation) (LTI) and physical stress (21 days forced swimming, have both physical and emotional component) (LTS), as well as to a long-term combination of these two stresses (LTI+LTS), both under basal conditions and in response to additional stressors – immobilization or cold. The activity of pituitary-adrenocortical and sympatho-adrenomedullary system was judged via the changes in plasma NA, A, ACTH and corticosterone (CORT) level.

Materials and methods

Adult rat males of Wistar strain, weighing 300–360g at the onset of experiments were used. The animals acclimated to 21 ± 1°C, with a constant day/night cycle (light on from 7.00 a.m. to 7.00 p.m.) had free access to standard laboratory rat chow and water. All procedures were performed during the light period between 9.00 a.m. and 2.00 p.m. Protocol of the "Vinča" Institute on care and treatment of laboratory animals was strictly followed. The rats were divided into four groups each group consisting of 8 animals. The first, naive control group consisted of four animals per cage. The second group involved the rats individually housed for 21 days. The third group was exposed to long-term forced swimming stress. The animals were housed four per cage and submitted to forced swimming everyday for 15 min in water heated to 32°C, during the 21-day-period. The rats of the fourth groups were individually housed and exposed to forced swimming under the same conditions as those of the third group. On the day before blood sampling, a cannula was inserted into the tail artery under pentobarbital (40 mg/kg i.p) anaesthesia. A cannula (PE 50; 0.75 m long, 0.58 mm in internal diameter) filled with a solution of 0.9% NaCl and containing 300 IU/ml sodium heparin was inserted into the ventral caudal tail artery. The cannula was tunneled under the skin and exited at the nape. A spring wire protected the catheter. After surgery, each rat was housed in an individual cage, with the protected cannula extending out of the cage with a 1 ml syringe at the end. Blood samples (0.5 ml) were collected via the cannula at the indicated times and the same volume of heparinized saline (50 IU/ml) was administered intraarterially after each blood sample was obtained. Repeated blood sampling using this protocol does not affect plasma levels of catecholamines [8]. This allowed the plasma catecholamines, CORT and ACTH level estimation without additional stressing of the animals during manipulations. After the baseline blood collection, the rats were immobilized or exposed to cold stress. Blood was collected 15, 30, 60 and 120 min after the onset of immobilization. The animals exposed to cold for 2 h, were kept initially at ambient temperature and after the baseline blood collection, carefully transferred within their home cages into the cold chamber (4°C) and the blood samples were collected 30, 60 and 120 min later.

Immobilization stress was induced as described by Kvetnansky and Mikulaj [9]. Plasma catecholamines were assayed by a modification of the radioenzymatic method described previously [10]. Catecholamines present in plasma aliquots were converted into their labeled O-methylated derivatives by S-[3H]adenosylmethionine and lypophilized catechol-O- methyltransferase isolated from rat liver. The O-methylated derivatives of the amines were then extracted along with unlabeled carrier compounds, separated by thin-layer chromatography, eluted and reacted with periodate.Plasma CORT content was measured directly upon prior extraction using RIA commercial kits (ICN, Biochemicals, Costa Mesa, CA, U.S.A.) and the concentration of CORT is expressed in ng /ml plasma. Plasma ACTH concentration was determined by chemiluminescent method using an IMMULITE automatic analyzer (DPC, Los Angeles,CA, U.S.A.) and the concentration of ACTH is expressed in pg /ml plasma.

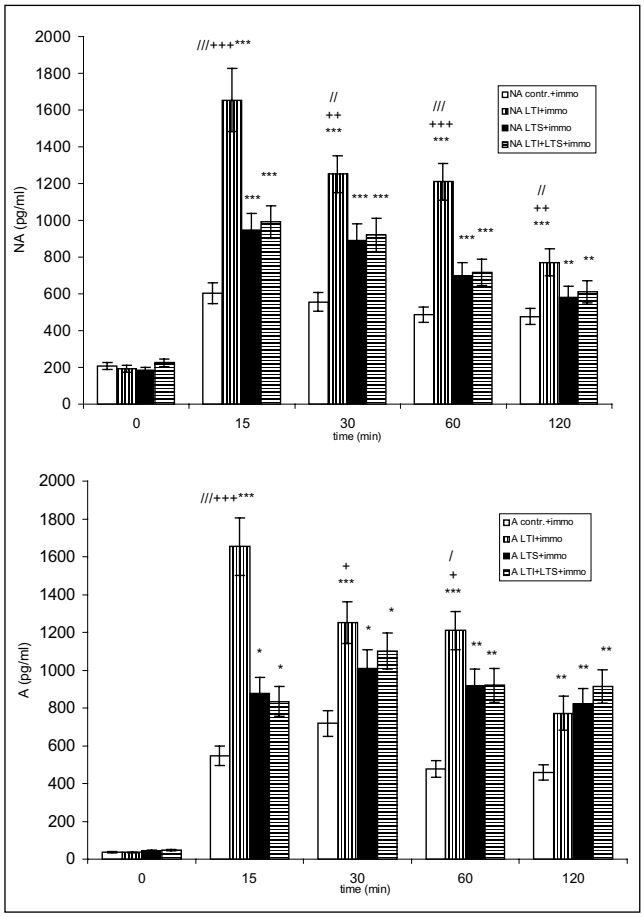

Figure 1. The effects of acute immobilization on plasma level of noradrenaline (NA)and adrenaline (A) (pg/ml) in the controls, rats exposed to long-term isolation (LTI), long-term forced swimming (LTS) and long-term isolation accompanied by forced swimming (LTI+LTS). The values are means ±SEM of 6-8 animals. Statistical significance *p<0.05, **p<0.01 and ***p<0.001 as compared to the control; +p<0.05, ++p<0.01 and +++p<0.001 LTI vs. LTS; /p<0.05,// p<0.01 and ///p<0.001 LTI vs. LTI+LTS.

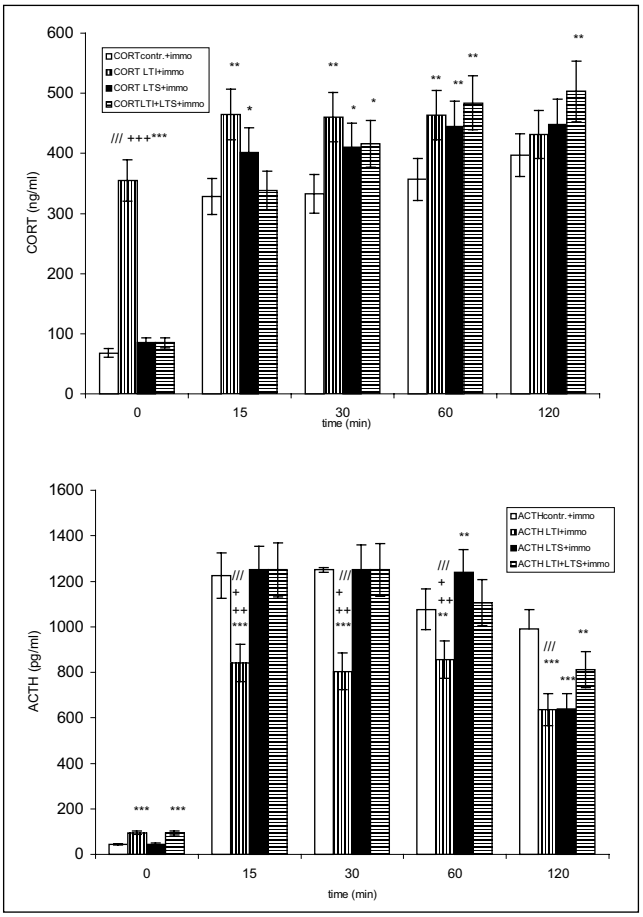

Figure 2. The effects of acute immobilization on plasma level of adrenocorticotropic hormone (ACTH) (pg/ml) and corticosterone (CORT) (ng/ml) in the controls, rats exposed to long-term isolation (LTI), long-term forced swimming (LTS) and long-term isolation accompanied by forced swimming (LTI+LTS). The values are means ±SEM of 6-8 animals. Statistical significance *p<0.05, **p<0.01 and ***p<0.001 as compared to the control; +p<0.05, ++p<0.01 and +++p<0.001 LTI vs. LTS; /p<0.05,//p<0.01 and ///p<0.001 LTI vs. LTI+LTS.

Statistical significance of the differences between the treated groups and the control were evaluated by one-way ANOVA test.

Results

Long-term exposure of adult rat males to either isolation or forced swimming, did not influence the basal plasma NA and A levels. However, exposure to immobilization produced an increase in plasma NA and A level in all investigated groups. In the LTI group, plasma NA and A levels were elevated in all experimental time points of immobilization. In LTS and LTI+LTS groups, immobilization also resulted in increased plasma levels of NA and A. This increase was higher than that found in the controls exposed to immobilization only, but lower than in the LTI group additionally subjected to immobilization (*Fig. 1*). Basal plasma ACTH levels were significantly elevated in LTI group and that exposed to combined stress, while basal plasma CORT levels were significantly elevated in LTI and to a lesser extent in LTS and combined stress groups. Immobilization elicited a conspicuous increase of plasma ACTH in the controls, LTS and LTI+LTS group, but somewhat lower increase was recorded in LTI group. Immobilization expressed a similar effect on CORT level in all investigated groups and its concentration was significantly elevated throughout the entire period of immobilization (*Fig. 2*).

Exposure of rats to 4°C for 2 h led to an increase in NA level in all groups of animals, the highest one being observed in LTI group. It can be seen that exposure to acute cold (2 h) resulted in an increased plasma level of A in all groups of animals, but this increase was not statistically significant. This slight elevation could be the result of transferring the animals into a cold chamber and other manipulations (*Fig. 3*). The acute 2-h-cold stress produced a significant increase of plasma ACTH content in LTS group and the group exposed to combined action of isolation stress and forced swimming. This increase was observed in the control group but it was lower, while a surprisingly low increase of this hormone level was recorded in LTI group additionally exposed to 2-h-cold stress. The cold produced a remarkable increase in plasma CORT concentration

of all long-term stressed animals as compared to the control (*Fig. 4*)

Discussion

Pijlman et al. [11] have recently reported that physical and emotional stress differed with regard to long-term effects on behaviour because the former resulted in inactivity in a small open field and the latter led to hyperactivity of experimental animals. Somewhat later, the same authors [12] found that physical stress induced a long-term decrease of both preference for saccharin and open field activity but emotional stress showed an increase in open field and a slight increase in saccharine preference. It has been concluded that response to an additional stress depends on stress modality.

The results presented here showed that the two long-term stressors of different characteristics applied in the present work expressed different effects on the level of plasma catecholamines, ACTH and CORT in rats. In rats exposed to LTI, as well as in those exposed to LTI+LTS, the basal plasma ACTH level was increased approximately two times, as compared to other groups. At the same time, basal plasma CORT content was enhanced several fold in LTI group, while only a slight increase was observed in the group of rats exposed to LTS, as well as in the group exposed to the combination of these two stresses. However, none of the treatments influenced basal plasma NA and A concentrations were not changed in all investigated groups. These results could be partly connected to the data of Miachon et al.[13] who showed that the 13-week-isolation resulted in a significant increase in catecholamine turnover in hippocampus, cortex and cerebellum, a certain increase in ACTH and a decrease in CORT levels. The explanation for the differences between our results and the data of Miachon et al. [13] could be related to the differences in the duration of the isolation. We decided to apply the isolation stress for 21 days, because several authors suggested that this period of stress was sufficiently long to induce long-lasting behavioural sequel associated with

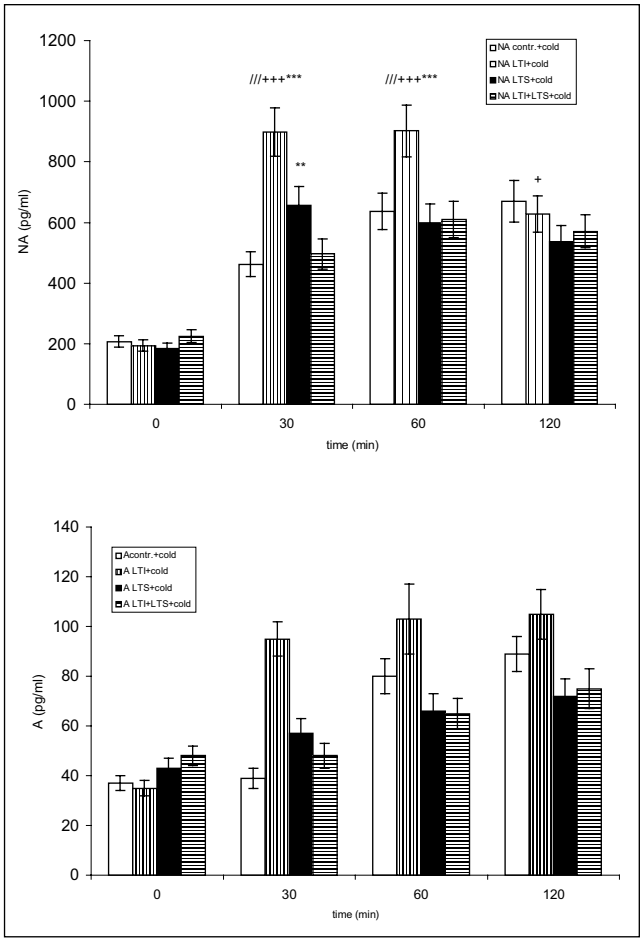

Figure 3. The effects of acute cold stress on plasma level of noradrenaline (NA)and adrenaline (A) (pg/ml) in the controls, rats exposed to long-term isolation (LTI), long-term forced swimming (LTS) and long-term isolation accompanied by forced swimming (LTI+LTS). The values are means ±SEM of 6-8 animals. Statistical significance *p<0.05, **p<0.01 and ***p<0.001 as compared to the control; +p<0.05, ++p<0.01 and +++p<0.001 LTI vs. LTS; /p<0.05,// p<0.01 and ///p<0.001 LTI vs. LTI+LTS.

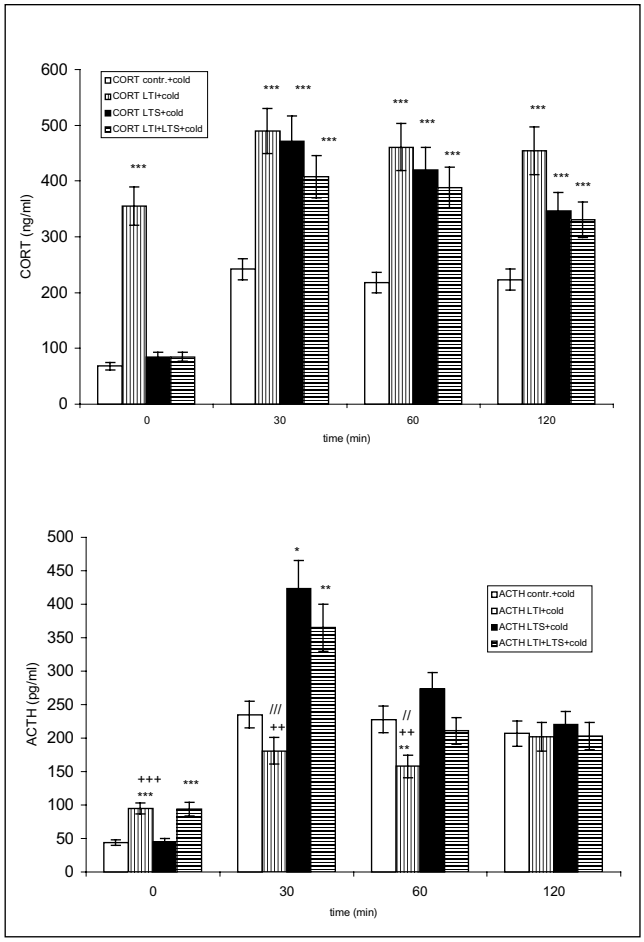

Figure 4. The effects of acute cold stress on plasma level of adrenocorticotropic hormone (ACTH) (pg/ml) and corticosterone (CORT) (ng/ml) in the controls, rats exposed to long-term isolation (LTI), long-term forced swimming (LTS) and long-term isolation accompanied by forced swimming (LTI+LTS). The values are means ±SEM of 6-8 animals. Statistical significance *p<0.05, **p<0.01 and ***p<0.001 as compared to the control; +p<0.05, ++p<0.01 and +++p<0.001 LTI vs. LTS; /p<0.05,//p<0.01 and ///p<0.001 LTI vs. LTI+LTS.

reproducible neurochemical and immunological modifications [14–16]. Our data shows that during long-term physical and psychosocial and combined stress the basal plasma catecholamine levels remained unchanged. It seems that only acute stress markedly activated sympatho-adrenal system and recovery after long-term stress. This is in agreement with the findings of Pol et al. [17] who reported that chronic exposure to immobilization stress did not alter NA in frontal cortex, hippocampus or hypothalamus, as judged by its content measured approximately 20 h after the last exposure to immobilization, but 2-h-immobilization resulted in a significant decrease of NA level in these brain structures.

Exposure of rats to long-term psychosocial, physical and combined stress and then to immobilization for 2 h led to the activation of both pituitary-adrenocortical and sympatho-adrenomedullary systems. The highest elevation of plasma NA and A was observed in LTI rats, somewhat lower increase was found in LTS group and LTI+LTS rats and the least one in control rats. Sgofio et al. [18] suggested that social defeat induced a much greater elevation in NA and A content, indicating a higher involvement of sympatho-adrenomedullary system. We found that an additional stressor such as immobilization led to the most conspicuous activation of sympatho-adrenomedullary system in the group exposed to LTI. Interestingly, LTI+LTS and LTS rats responded to immobilization as a heterotypic additional stressor by less significant activation of sympatho-adrenomedullary system comparing to LTI group. It seems that forced swimming (15 min daily for 21 days) combined with long-term isolation attenuated the enhanced activity of sympatho-adrenomedullary system elicited by the action of novel stress. The data of Haller and Halasz [19] showed that when isolated rats were daily exposed to short-term defeats, the anxiogenic effect of isolation was completely abolished. They wondered about whether a mild daily stressor would abolish the neurochemical effects of isolation. Our results suggested that 15-min- swimming for 21 days in long-term isolation rats activated sympatho-adrenomedullary system to a lesser extent when exposed to novel stressors than in rats exposed to long-term isolation only.

Immobilization produced a significant increase of plasma ACTH content in LTS and the rats exposed to combined stress, and somewhat lower increase in LTI animals. Plasma CORT level in LTI, LTS and the group that suffered the combination of the two stresses was also increased and no significant differences were found between these three groups. This is in accordance with the data of Moura and Moares [20] and Rittenhouse et al. [21] who reported that forced swimming resulted in an increased plasma ACTH and CORT levels. Our results showed that long-term psychosocial stress followed by a additional stressor, e.g. immobilization provoked a strong response of sympatho-adrenomedullary system and somewhat weaker response of pituitary-adrenocortical axis.

Short-term exposure to cold of the rats that suffered long-term isolation, forced swimming or the combination of the two stresses, led to a pronounced activation of both sympathoneural and pituitary-adrenocortical axis, but not of adrenomedullary system. These findings could be related to the data of Vollmer et al. [22] who found that the concentration of NA was significantly increased during 24 h of cold stress (4°C), while the level of A remained unchanged. The exposure of all three groups of rats in our experiments to acute cold was followed by significantly elevated levels of CORT. Cold produced an increase in plasma CORT content in unstressed naive controls, as well, but this increase was lower comparing to that observed in long-term stressed rats. This is in agreement with the findings of Hashiguchi et al. [23] who suggested that CORT is especially sensitive to the action of a new stress. The additional cold stress provoked the highest elevation of plasma ACTH level in LTS (about 11-fold), lower increase in combined stress (about 5-fold) and the lowest in LTI (about 2-fold) group. These results show that although LTI resulted in a significant elevation of the basal plasma ACTH level, exposure to additional stressor produced the lowest increase of this hormone content in comparison with that recorded in LTS and LTI+LTS group.The present study demonstrates that immobilization produced a higher increase in plasma catecholamines, ACTH and CORT levels in chronically stressed rats comparing to cold stress. Therefore, it is obvious that the response of the animals additionally exposed to short-term action of additional stressors depended on the type of stressor.

Based on these results, it may be concluded that long-term isolation as a psychosocial stress acts as a stronger stressor than long-term forced swimming stress. Our results are consistent with the data of Endo et al. [24,25] who found that long-term exposure to psychological, but not physical stress, caused a significant elevation of body temperature, probably due to an increase of sympathetic tone. The same authors also found that psychological stress could have a weaker influence as an acute stress, but in the case of repeated exposures, the effects of psychological stress could grow larger or persist for a longer period of time, as compared to physical stress. Based on the results obtained throughout the present study it seems that repeated short-term forced swimming attenuate the effect of long-term isolation on the activity of sympatho-adrenomedullary system. Radak et al. [26] investigated effects of immobilization, a single bout of exercise and immobilization followed by exercise on oxidative damage of macromolecules in hippocampus of rat brain. They found that the oxidative damages of lipids, proteins and nuclear DNA were significantly increased in immobilization group, while no increase was observed after a single bout exercise and immobilization followed by exercise. It appears that short-term daily swimming expressed a protective effect against the effects of long-term isolation.

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