EFFECTS OF EXPOSURE TO SPACE FLIGHT ON ENDOCRINE REGULATIONS IN EXPERIMENTAL ANIMALS

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This minireview summarizes the results of the observations on changes in endocrine functions of rats exposed to space flights for various periods. The results found after space flights are compared with those obtained from rats in acute or repeated restrain stress. A slight increase of plasma catecholamine levels was observed in rats after space flight of longer duration (>14 days), but no changes in catecholamine content in the activity of catecholamine synthesizing enzymes were noted in adrenal medulla and in hypothalamus. The norepinephrine content was, however, decreased in several nuclei selected from hypothalamus of flight rats. Plasma corticosterone levels were increased after space flight and morphological examination of pituitary showed elevated activity of corticotrophs. However, the plasma levels of ACTH were not increased in rats 6 hours after space flight. These changes in plasma hormone levels affected the activity of enzymes involved in metabolism of amino acids in liver and lipolysis in adipose tissue. The plasma levels of testosterone and triiodothyronine were diminished after space flight suggesting the suppression of the thyroid and gonadal activity. Increase of plasma insulin and glucose levels were found in rats after space flight, but the glucagon values were not changed. Comparing these results from flight rats with the animals exposed to acute or repeated stress indicate that long stay in microgravity do not represent very intensive stressogenic stimulus for adrenocortical and sympatho-adrenomedullar systems, and hormone alterations observed after space flight may be due to acute gravitational stress resulting from a return to Earth gravity. Therefore further studies including the inflight animal experiments on a board of International Space Station are necessary for elucidation of the effects of microgravity on endocrine functions.

Key words: Space flights – Rats – Adrenal glands – Thyroid function – Sympatho-adrenomedullary system – Gonads.

The space flights of human subjects and of experimental animals are associated with several stress factors including microgravity, which affect the function of endocrine glands (LEACH et al. 1976, 1983; NOSKOV et al. 1981; KVETNANSKY et al. 1988; MACHO et al., 1991). The understanding of the influence of stress loads and inflight activity of astronaut at altered gravity conditions, on the physiological functions of human and animal organism, is important for evaluation of the capacity of mammalian organism to cope with short or long-term space flights and with postflight adaptation to Earth's gravity. The inflight exposure of astronaut to physical, metabolic and psychical loads showed the differences in the response of neuroendocrine regulatory system to these stressors in comparison to preflight results (KVETNANSKY et al. 2000; MACHO et al. 2001). Simultaneously with human space flights several series of experiments using rats exposed to space flights (Table 1) on board of special BION – COSMOS satellites or Shuttle Transportation System (STS) were performed. The aims of these experiments were to

Table 1
Selected space flights with rat studies of plasma hormones, tissue enzymes and metabolism.*

Mission	Length of flight (days)	Year
COSMOS 782	19.5	1975
COSMOS 936	18.5	1977
COSMOS 1129	18.5	1979
STS 8	6	1983
COSMOS 1514	5	1983
STS 10	10	1984
COSMOS 1667	7	1985
STS 51B	7	1985
COSMOS 1887	12.5	1987
STS 29	5	1989
COSMOS 2044	14	1989
STS 41	4	1990
STS 40 (SLS – 1)	9	1991
STS 48	5	1991
STS 46	8	1992
STS 52	10	1992
STS 54	6	1993
STS 56	10	1993
STS 57	10	1993
STS 58 (SLS – 2)	14	1993
STS 60	8	1994
STS 62	14	1994
STS 65	15	1994
STS 63	8	1995
STS 77	10	1995
STS 78	17	1996
STS 80	18	1996
STS 90	16	1998

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analyze the mechanisms of the changes in endocrine functions during space flights and to evaluate the role of hormonal changes in metabolic processes, bone and muscle atrophy and in alteration of immune system function (Nemeth et al. 1981; Popova and Grigoriev 1994; Ilyin et al. 2000). These animal studies are very important to try and verify some hypotheses and to confirm the results obtained in the still too few astronauts available for medical observations during space flights. In animal experiments it was possible not only to evaluate plasma hormone levels but also to study morphological alterations in the organs, enzyme activities in tissues, metabolic processes and their hormonal regulations. The disadvantage, however, was that rats in almost all experiments could be examined only after the space flight or during the postflight period, meaning that actual inflight changes could not be measured. In this review, attention is concentrated on the evaluation of the changes in endocrine system of rats exposed to space flight for various periods and comparison of the neuroendocrine regulation to other kinds of stressors under normal gravity. The special efforts will be paid to our results obtained from COSMOS experiments and to evaluation of these data in relation to those obtained on STS missions. The facilities on the research sections of International Space Station (ISS) will allow performing the inflight animal observations of endocrine system functions at various times of space flight. This will open the possibilities to study in more details the function of endocrine glands during the inflight period and to evaluate direct effects of microgravity on animal organism.

Effect of space flight on sympathetic-adrenomedullary system activity

The state in microgravity is an extremely non-physiologic situation, which living organism has never encountered on Earth, and it may act as a stressor. The sympathetic-adrenomedullary system is highly activated at the exposure of an organism to various stressors (Kvetnansky and Mikulaj 1970; Kvetnansky et al 1978, 1981). Therefore the activity of this system was studied in human subjects (Kvetnansky et al. 1991; Leach and Rambout 1977) and in rats (Kvetnansky et al. 1983; Macho et al. 1996) exposed to space flights for various periods. The results of present observations showed that plasma epinephrine (E) concentrations, determined 6 hours after landing (COSMOS 782,936,1129,1514), were similar in control and in rats exposed to space flight for a short period (5 and 14 days). However, significant elevations of plasma E were seen when E values in flight rats were compared to E levels in synchronous model groups after space flight of longer periods (18.5 and 19.6 days). Plasma norepinephrine (NE) concentrations were increased 6 hours after space flights in all experiments (Fig.1). Similar increases in plasma norepinephrine levels were observed in rats exposed to artificial gravity during space flight on a board of space satellite (Kvetnansky et al. 1981a,b). No differences in plasma cate-

cholamine content between animals exposed to space flight and controls were observed after 25 days of postflight readaptation period (TIGRANIAN et al. 1982).

There were no changes in the content of E and NE in adrenal tissues of animals exposed to microgravity or in rats subjected to artificial gravity in centrifuge on a board of satellite during 18.5-day space flight (TIGRANIAN et al. 1982). The determination of catecholamine synthesizing enzyme activities showed a slight increase of tyrosine hydroxylase (TH) activity after 19.5-day space flight, but no changes were noted in any other flights (COSMOS 936 and 1129, KVETNANSKY et al. 1980, TIGRANIAN et al. 1982). The activities of dopamine-beta-hydroxylase (DBH) and phenylethanolamine-N-metyl-transferase (PNMT) in adrenal tissues were similar in flight rats and control animals. The adrenal catecholamine content and also the activities of catecholamine synthesizing enzymes were similar after 25-day postflight readaptation period in control rats and animals exposed to space flights (KVETNANSKY et al. 1981a).

Activation of the sympathetic-adrenomedullary system and pituitary-adrenocortical system is a result of complex neuroendocrine reactions, in which brain catecholamines are also involved. The brain adrenergic system, particularly in the hypothalamus has been found to be activated under stress (KVETNANSKY et al. 1983). Therefore the influence of stressful factors of the space flights on the activity of catecholaminergic systems in brain, mainly in the hypothalamus was also evaluated. The catecholamine concentrations in hypothalamus of rats exposed to space flights (COSMOS 782, 936 and 1129) were similar in animals in microgravity or in an artificial gravity (KVETNANSKY et al. 1983). No significant differences were noted in catecholamine synthesizing enzyme activities in hypothalamus of flight rats and those from synchronous model or control groups. The catecholamine content was also determined in six-isolated hypothalamic nuclei and a reduced content of NE was found in the arcuate nuclei, periventricular nuclei and in median eminence. The content of E was diminished in median eminence, in perivetricular and suprachiasmatic nuclei. The content of dopamine in hypothalamic nuclei was not affected by space flight (KVETNANSKY et al., 1983). In agreement with the observation on the content of catecholamines in hypothalamus no changes in NE content were found in selected areas of the brainstem of rats after 14-day space flight (SLS-2). However, a decrease of NE content was observed in locus coeruleus after 9-day SLS-1 mission. This was explained by stressful conditions at landing on return to Earth (FAREH et al. 1993; FAGETTE et al. 1996)

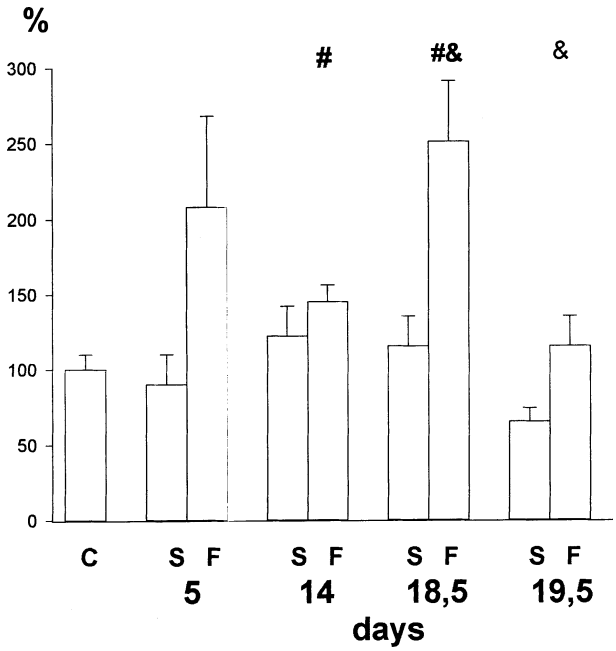

Fig 1 Plasma norepinephrine levels in rats exposed to space flights. Values in flight rats (F) and in sychron-model groups (S – animals in laboratory in the same conditions as flight rats except microgravity) are expressed as percentage of control values (C).Flight duration in days. Differences are significant # = F:C P<0.05, & = F:S P<0.05

The changes of catecholamine plasma levels, catecholamine content in adrenal medulla and hypothalamus and in the activities of enzymes involved in catecholamine synthesis in adrenal and hypothalamic tissues observed after space flights were compared with the effects of acute or repeated stressors in rats. The first exposure of rats to forced immobilization, an acute stress, results in a high increase of plasma E and NE with a decrease of catecholamine especially E, content in adrenal medulla (KVETNANSKY et al. 1983, KVETNANSKY and MCCARTY 2000a). The activities of enzymes involved in catecholamine synthesis – TH, DBH and PNMT – were unchanged or slightly elevated immediately after acute stress ex-

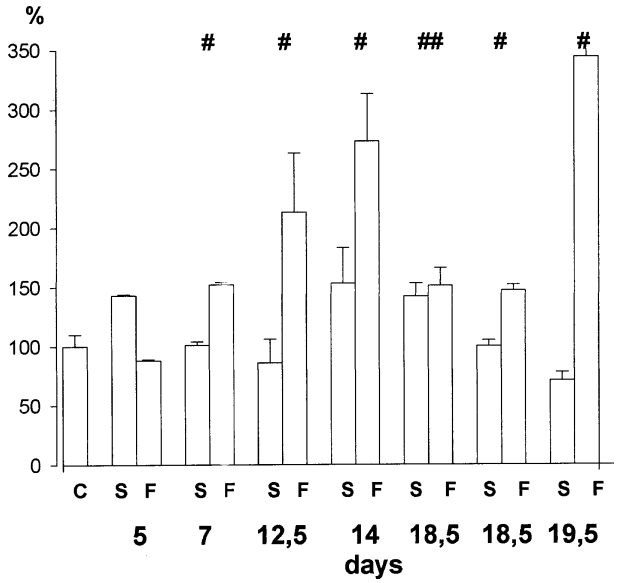

Fig 2 Plasma corticosterone levels in rats after space flight for various periods. Values in synchron-model groups (S) and in flight rats (F) are expressed in percentage of control levels (C). Flight duration in days. Differences are significant # F:C , S:C, P<0.05

posure, however, the transient increase of transcriptional activities for these enzymes (measured by mRNA levels for TH, DBH and PNMT) was noted (KVETNANSKY and MCCARTY 2000b). In animals repeatedly exposed to immobilization stress (6 or 42 days, 150-min daily) increased basic values of plasma E and NE were noted. Elevation of plasma catecholamine levels and increased activity of catecholamine synthesizing enzymes in adrenal medulla (KVETNANSKY et al. 1979) followed the exposure to immobilization of repeatedly stressed rats. Compared to unstressed control rats, the animals exposed to immobilization for 6 consecutive days have significant elevations in adrenal medullar mRNA levels of TH, DBH and PNMT (KVETNANSKY and MCCARTY 2000b). Elevated enzyme and transcription activities persist for at least 24 hours after end of stress. Thus the adrenal medulla of chronically stressed animals exhibits a significant and long lasting enhancement of catecholamine biosynthetic capacity. However, in animals subjected to space flights both in microgravity and in artificial gravity on a board of satellite, the catecholamine content in adrenal medulla showed no decrease and the activities of catecholamine-synthesizing enzymes were not elevated, suggesting that the prolonged stay in microgravity and landing, do not act as an intensive stressor on the sympathoadrenal system as do repeated immobilization or trauma (KVETNANSKY et al., 1983).

In hypothalamus an acute immobilization stress induces a significant decrease of both NE and E content in the majority of hypothalamic nuclei, whereas repeated exposure to this stressor resulted in highly significant increase of NE in all the hypothalamic nuclei studied, and in increased concentrations of dopamine. The content of NE tends to decrease in the majority of hypothalamic nuclei from flight rats, which is typical manifestation of acute stress (KVETNANSKY et al 1983). The activities of TH and DBH in hypothalamus are highly increased in rats exposed to repeated stress. But in the rats subjected to space flight no changes in TH or DBH activities in hypothalamus were noted, which suggests that catecholamine synthesis does not increase beyond the space flight as it is after chronically stressed rats. The evaluation of these data on catecholamine levels in plasma, adrenal medulla and hypothalamus, and on the activities of catecholamine synthesizing enzymes in adrenal and hypothalamic tissues in rats exposed to space flight demonstrate that long-term exposure of rats to microgravity does not represent a potent stressogenic stimulus on sympathetic-adrenomedullary system (KVETNANSKY et al., 1978, 1983). The most likely interpretation of the changes of catecholamine and their synthesizing enzyme activities determined in adrenal and hypothalamic tissues of rats after space flights appears to be the effects of an acute stressor acting during the landing maneuver. Therefore new approaches for evaluation of sympathoadrenal system activity among astronauts and experimental animals before, during and after space were devised to evaluate not only the plasma levels of catecholamines but also their rate of synthesis, release, spillover, uptake, reuptake and degradation (KVETNANSKY et al., 1994). In future experiments it will be necessary to use the facilities for animal experiments on ISS for direct observations and collection of tissues from rats during the stay in microgravity and compare these samples with those obtained several hours after flights.

Space flights and the activity of hypothalamic-pituitary-adrenocortical function

The activation of hypothalamic-pituitary-adrenocortical axis (HPAA) is usual response of the organism to various stressors (Nemeth et al. 1977). Therefore the changes in the HPAA were repeatedly studied after exposure of human subjects or experimental animals to acute or chronic stress stimuli. Plasma levels of corticosterone were markedly increased in rats exposed to space flight (of various duration ranking from 7 to 19.5 days (COSMOS 782, 936, 1129, 1667, 1887 and 2044, Fig.2), when compared to animals in synchronous model experiment (SM). This increase may have been due to the late effects of microgravity in addition to some gravitational stress from the return to 1-g gravity at landing. It was interesting that the exposure of rats to artificial 1 g gravity during the space flight in a small centrifuge on a board of space satellite (COSMOS 936, 18.5 days) eliminated this augmentation of plasma corticosterone (Tigranian et al. 1981; Macho et al. 1991). No differences in plasma corticosterone concentrations were observed after 6 and 25 days of readaptation period (Tigranian et al. 1981).

In spite of the elevated corticosterone plasma levels, no significant differences in ACTH plasma values were noted in flight rats and in animals in synchronous model experiments (Table 2) 6 hours after flight or after period of readaptation (Tigranian et al. 1982). However, the morphometry, immunocytochemistry and in situ hybridization of rat pituitary revealed a marked enlargement of corticotrophs with strong indication of increased secretory activity and enhanced expression of proopiomelanocortine mRNA (Thapar et al. 1994). The morphological findings reflect a hypersecretory state of corticotrophs, which is consistent with repeatedly observed increase of plasma corticosterone in rats exposed to space flight for varying periods. Also the increase of absolute and relative mass of adrenal tissues, noted in flight rats, reflects the hypersecretory activity of corticotrophs. The results of the measurement of corticosterone content in adrenals and hormone release from adrenal tissue incubated in vitro showed elevated adrenal corticosterone content in flight rats, but the release was similar as in control animals. Also the response to ACTH was comparable in control and flight rats (Table 3). Similar results were observed in animals, which were several hours after the repeated immobilization stress (Macho et al. 1980) suggesting presence of mild chronic stress in animals exposed to space flight. The increased activity of HPAA for a longer time in microgravity, besides the morphological changes in pituitary and adrenals, is also supported by estimation of the activities of enzymes involved in metabolism of amino acids in liver. The activities of enzymes rapidly responding to increased corticosterone during acute stress like tyrosine aminotransferase, tryptophan pyrrolase and serine dehydratase are increased after space flight (Nemeth et al. 1981). However, an augmentation of alanine aminotransferase (ALS) and

Table 2
Pituitary hormone levels in plasma and anterior pituitary tissue of rats after space flight.

PLASMA				
Hormone	Flight (days)	Control group	Synchron group	Flight group
ACTH (ng/ml)	18.5	290±22	230±32	238±30
TSH (mU/ml)	18.5	61±20	133±42	8±3 *

PITUITARY				
Hormone	Flight (days)	Control group	Synchron group	Flight group
ACTH ng/mg	18.5	173±30	105±16	131±70
TSH ng/mg	18.5	130±16	140±8	128±12
LH ng/mg	18.5	21.8±1.2	20.7±2.0	21.5±3.5
FSH ng/mg	185	11.0±0.58	10.2±0.7	9.2±1.5

Difference is significant * C:F p< 0,05, means ±SE,
for explanation of groups see Table 4.

Table 3
Corticosterone production by rat adrenal tissue incubated *in vitro* after the exposure to space flight (18.5 days) and the effect of ACTH

Corticosterone	Control	Synchron	Flight
Basal production*	3.23±0.22	3.62±0.22	2.44±0.61
ACTH – 25 mU/ml	4.91±0.44^{+}	4.69±0.39^{+}	4.51±0.54^{+}

*Production of corticosterone (µg /100 mg of adrenal tissue per 1 hour);
$^{+}$ stimulation with ACTH is significant (P<0.05), means±SE;
for explanation of groups see Table 4

Table 4
Testosterone production by rat testes after exposure to space flight (18,5 days).

Testosterone	Control	Synchron	Flight
Basal production	88,5±13,5	83,6±12,2	51,8±9,5 #
Stimulation with LH	180,4±26,9	184,3±21,2	123,8±18,6 #

Testosterone production in μg/100 mg per 3 hours.
LH was added in a dose of 4 μg/2 ml of incubation medium.
Control group – rats in vivarium,
Synchron – animals in the same conditions as flight rats except microgravity;
Fflight – group of examined 6 hours after flight.
= flight to control P<0.05. Means±SE.

aspartate transferase (AST) activity in liver was repeatedly demonstrated in flight rats (Németh et al. 1981; Macho et al. 1991), suggesting that the period of elevated corticosterone plasma levels was not limited only on the period of landing, but the hypercorticosteronemia was present also several days before the end of space flight. These enzymes (ALT, AST) are so called slowly responding enzymes to corticosterone requiring for an activation an elevated corticosterone levels for several days as it is usually during the exposition of animals to repeated stress load (Nemeth et al. 1977).

Comparison of the changes of plasma corticosterone and of hormone production in flight rats and in animals exposed to other stressors (immobilization, hypokinesia) showed that postflight increases of corticosterone are relatively smaller than those after an immobilization stress (Macho et al. 1980).

The presence of continuous stress effects on HPAA during space flight may be also supported by the results of the exposure of flight rats to other kind of stressogenic stimuli. In animals repeatedly exposed to one kind of stress an exaggerate response of plasma corticosterone was noted at the application of novel stress stimuli (Macho et al. 1980). It was interesting to note, that the exposure of flight rats to novel stressogenic stimuli – immobilization stress – during the postflight period results in an exaggerate elevation of corticosterone (Macho et al. 1996).

The studies of HPAA activity showed that the exposure of rats to microgravity and gravitational stress at landing was not very potent stressogenic stimuli. In spite of slight elevation of plasma corticosterone levels, the metabolic effects of this hormone were still strong enough to induce the changes in liver enzyme activities, in bone and muscle tissues (Nemeth et al. 1977, Kumei et al. 1998). It is possible that increased sensitivity in these tissues to corticosterone may be due to changes of the binding capacity of specific receptors for this hormone in the target tissues (Kumei et al. 1998).

Effects of space flight on gonadal function

The hypothalamic – pituitary – gonadal function is inhibited after exposure of human subjects and experimental animals to several stressors. This stress dependent phenomenon, mostly mediated by neural mechanism, results in decrease of plasma levels of luteinizing hormone in men and consequently in diminished production of testosterone and other androgens in testis (Strollo 1999). An impairment in androgen production has been found in human subjects during space flight. The testosterone levels were decreased in various biological fluids (plasma, saliva and urine; Strollo et al. 1998) in 7 astronauts during space flight, while a slight increase of LH values in plasma was noted as compared to preflight levels. Possible explanation for decreased testosterone values and non adequate diminution of LH might be the primary testicular disturbances. Due to typical pulsatility of pituitary hormone secretion, including LH, the increase plasma level of LH in one sample probably does not reflect the actual secretion pattern of this hormone (Strollo et al. 1998).

The investigation of testicular endocrine function in rats after space flight showed a small decrease of relative mass of testes and also testosterone plasma levels after 19.5 – day space flight (Tigranian et al.1982). Similar results with significant plasma testosterone decrease were observed in rats after 14 day space flight (Merill et al.1992). A decrease of androgen production was observed during real and simulated weightlessness (Amann et al. 1992; Hadley et al. 1992). The diminution of testosterone production by rat testis after exposure to 18.5-day spaceflight was observed. The stimulatory effect of LH on testosterone release was decreased (Table 4). This lower response of testicular tissue to LH supports the explanation that the decreased testosterone levels are due to primary testicular disturbances (Strol-

LO et al. 1998). When tail suspension (hind limb suspended rat) was used to simulate weightlessness conditions a significant reduction in plasma testosterone levels by day 6 and 12 of suspension was found. It was suggested that these decreases in testosterone values are, at least in part, responsible for muscle atrophy and for loss of mineral density of weight-bearing bones (WIMALAWANSA and WIMALAWANSA 1999). However ROYLAND et al. (1994) were not able to confirm the diminution of testicular function during three-week long simulation of microgravity.

The measurement of FSH and LH content in pituitary showed that there are no changes in these hormones after space flight (Table 2). However, the data on plasma FSH and LH levels in rats after space flight are still not available, therefore remains unknown whether the decreased testosterone levels reflect the changes in LH secretion, receptor or postreceptor deficit or other factors are involved. The dissociation between plasma LH and plasma testosterone level appears to exists during the exposure to altered gravity. The pulsatile release of LH and diurnal variability of testosterone secretion may mask the influence of gravity on pituitary gonadal axis when analyzing the limited samples of plasma for hormone concentrations. Therefore the whole day urinary excretion of LH and testosterone in rats exposed to space flight for 14 days and to increased gravity upon the return to Earth with animals exposed to elevated gravity by centrifugation at 2g was compared (ORTIZ et al. 2000). Increasing LH and testosterone excretion on the first 3 days of postflight period was noted. The values of excreted LH and testosterone were significantly correlated. A sustained increase of testosterone excretion during the exposure to centrifugation at 2g, (simulation of gravity effects on rats during the readaptation period after space flight) was observed which suggests that pituitary – gonadal axis of rat may adapt very quickly to increased gravity in postflight period. However, elevated excretion of testosterone was observed also in rats between days 1 and 8 of centrifugation, while excreted LH was reduced on days 2 and 3. The similar increase of testosterone excretion in postflight period and in animals exposed to centrifugation at 2g suggests that pituitary gonadal axis in flight rats may adapt very quickly to increased gravity at landing.

In spite of several woman astronauts no studies are available on their hypothalamo-pituitary-gonadal functions. There were two experiments with female pregnant rats exposed to space flight for 5 days (COSMOS 1514) or 9 and 11 days (STS 40, 66) during pregnancy (SAVELEV et al. 1988; MACHO et al. 1993; RONCA and ALBERTS 2000). No effects of space flight were noted on pituitary and ovary mass post partum (one day after landing, BURDEN et al. 1997). Space flight for 11 days did not alter follicular populations, fetal wastage in utero, plasma concentrations of progesterone and LH. A significant increase of plasma FSH was noted at the postpartum sampling time. However, the pituitary content of FSH was not changed, the content of LH was decreased. It was concluded that the space flight and exposure of pregnant rats to microgravity during the period of pregnancy is compatible with maintenance of pregnacy and has minimal effects on postpartum hypophyseal and ovarian parameters (BURDEN et al. 1997; MACHO et al. 1993). The flight experiments with pregnant female rats showed that fetuses can grow and develop when maternal organisms are exposed to microgravity (SAVELEV et al.; 1988, BURDEN et al. 1997).

The influence of space flight on hypothalamic pituitary thyroid function

The hypothalamo-pituitary-thyroid axis is stimulated mainly by cold stress, however, other stressogenic factors do not affect in great extend the thyroid function. In human subjects elevated plasma levels of thyroxin and diminished triiodothyronine values were noted during and after space flights (SHEINFED et al. 1975; LEACH et al. 1977; STEIN et al. 1999).

In rats exposed to space flights, plasma thyroxin levels were unchanged 6 hours after space flight in one experiment and they were lower in 2 experiments as compared to control groups (Fig. 3). Plasma levels of triiodothyronine were similar in flight and control rats, both 6 hours and 25 days after space flight, but marked increases in plasma levels of both thyroid hormones were repeatedly observed in synchronous model groups (Fig.3). Comparing the thyroid hormone values in flight rat and in synchronous model groups showed that exposure to microgravity diminished the thyroid hormone plasma levels. This

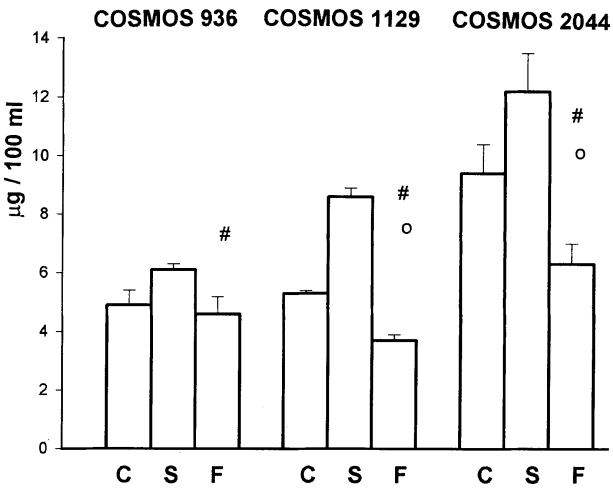

Fig 3 Plasma levels of thyroxine in rats after space flights. C – controls, S – synchron-model group (for explanation see Fig.1), F – flight rats. Differences are significant # S:F P<0.05, o C:F P<005

is in agreement with changes observed in human subjects and in monkey (Grindeland et al. 2000). The causes of the increase of thyroid hormones in plasma of synchronous model groups and in the differences of hormone changes between the experiments (unchanged thyroxin levels in rats from COSMOS 936, decreases in COSMOS 1129 and 2044), remain unclear. Further studies by using blood sampling during the flight are necessary to obtain the enough data for explanation of thyroid function in microgravity.

The morphological examination of thyroid glands from rats exposed to space flight (SLS-1, 9 days) showed histological alterations indicating a reduction of thyroid activity (Loginov 1994), and the presence of mild hypothyroidism in flight rats was suggested (Strollo 1999). The determination of the rate of thyroid hormone synthesis in rats after space flight for 5 days (COSMOS 1415) and 18.5 days (COSMOS 936), showed that 6 hours after landing there were similar percentage values of thyroxine and triiodothyronine in flight and control rats. But the ratio of hormone precursors (monoiodotyrosine and diiodotyrosine) to the thyroxine and triiodothyronine (which reflects the rate of thyroid hormone biosynthesis) was increased suggesting the accumulation of precursors in thyroid tissues of flight rats as compared to controls (Knopp et al. 1983). The content of thyroxine and triiodothyronine per unit of thyroid tissue mass was lower in flight rats as in synchron model groups. This indicates that there is a lower rate of thyroid hormone biosynthesis in rats exposed to spaceflight.

The morphological examinations of thyroid gland showed histological alterations in thyroid tissue indicating a reduction of thyroid activity in flight rats (Loginov 1994)

There were differences in TSH plasma levels from various experiments. In two experiments plasma TSH dropped in flight rats (COSMOS 782 and 1887, TABLE 2) in other a high levels of TSH were noted (COSMOS 936). An increase of TSH release was expected at lower levels of thyroxin induced by feedback mechanism. However, no changes in the content of TSH in pituitary gland were found in rats after space flight. No morphological alterations in pituitary thyrotrophs were observed in flight rats (Thapar et al. 1994). Until now there are no data on hypothalamic TRH release after space flight so the changes in the regulation of TSH secretion at the exposure to microgravity can not be fully explained. It was suggested that high CRH and glucocorticoid levels (which are typical for chronic stress) could blunt both TSH and thyroxin release thus maintaining relatively low thyroxin levels without concomitant increase in TSH (Strollo 1999). This is in agreement with the measurement of TSH content and morphological examination of pituitary thyrotrophs in rats after space flight (Tigranian et al. 1982; Thapar et al. 1994). Further studies of hypothalamo – pituitary – thyroid function are necessary to clarify the effects of microgravity on the thyroid function. Morphological data suggest a tendency toward decreased secretory activity in thyroid cells during the period immediately after space flights.

Hormone regulation of metabolism after space flights

Changes in the body fluid distribution, in unload of skeletal muscle and in plasma hormone levels during and after spaceflight induce alteration in metabolic processes in several tissues.

Because catecholamines have an influence on lipolysis in adipocytes, the plasma nonesterified fatty acids (NEFA) and the activity of lipolytic processes in adipose tissue were investigated in rats after ex-

posure to space flight. A small increase of NEFA was noted in plasma of flight rats (410±20 μmol / l in control and 511±30 μmol / l in flight rats). Slight increase or no changes of NEFA release from adipose tissue incubated in vitro was observed after space flight. However, the stimulatory effect of norepinephrine was significantly lower in rats exposed to space flight as compared to control (Macho et al. 1991). After 6 or 21 days of recovery period basal lipolysis was similar in control and flight rats, but the stimulatory effect of norepinephrine on lipolysis was still lower in flight animals as compared to control group (Macho et al. 1982). The activity of lipoprotein lipase in adipose tissue was increased in rats exposed to space flight for 18.5 days as compared to animals during the flight in artificial gravity (Skottova et al. 1982). The preflight values of lipoprotein lipase activities and lipolysis were observed on the 25 –day after flight. These results suggest that there are important changes in hormonal regulation of metabolism in adipose tissue.

The changes in plasma corticosterone levels influenced he activity of enzymes involved in the metabolism of amino acids in liver. (Nemeth et al. 1981). As it was mentioned above the activity of enzymes responding to acute elevation of corticosterone (tyrosine aminotransferase, tryptophan pyrrolase and serine dehydratase) is increased in rat liver after space flight. However, an augmentation of the activity of alanine aminotransferase and aspartate aminotransferase in liver was also repeatedly demonstrated. These enzymes are requiring for increase of their activities daily administration of corticosterone for several days or exposure of animals to repeated stresses (Nemeth et al. 1981). These results suggest the longer period of increase corticosterone plasma levels in flight rats. High accumulation of glycogen in liver was repeatedly observed in flight rats and this was explained by gluconeogenic affects of elevated glucocorticoids (Nemeth et al. 1981; Merrill et al. 1992; Popova and Grigoriev 1994).

Measurement of plasma insulin and glucose levels in astronauts showed significant changes in insulin levels during space flights and in the first days of recovery period (Leach 1983; Popova et al. 1983; Leach et al. 1991; Smirnov et al. 1991). It was reported that insulin plasma levels are elevated in cosmonauts on the first day of recovery period after short term (5-8 days) or long term (4-5 months) space flights (Leach 1983; Afonin 1989). However, during the inflight period, the plasma insulin levels were increased or unchanged in first few days, but between days 20-60 of space flight both insulin and glucose levels in plasma were decreased (Leach and Rambaut 1977; Leach 1983). Elevated insulin plasma levels were noted also during the oral glucose tolerance test performed on the 4th inflight day of Slovak astronaut and also in postflight period (Macho et al. 2001). Increased plasma insulin and glucose levels were repeatedly observed in animals experiments during the first hours after space flights (Fig. 4) for 7 to 19,5 days. When rats were exposed to artificial gravity during the space flight, the elevation of insulin was very slight and insignificant as compared to control. This suggests that insulin levels were affected predominantly by gravitational changes and other factors of space flights or landing maneuvers have little effect. Blood glucose levels did not showed a corresponding decrease to the insulin plasma values during this recovery period suggesting that the space flight produced a decreased sensitivity to insulin in the organism. The delayed utilization of glucose after a glucose load in astronauts was observed during short term (Macho et al. 2001) or long term spaceflight (Vorobyov et al. 1984). To analyze the mechanism of decreased insulin sensitivity the first step of insulin action, the binding of insulin to receptors in target tissues was studied. No significant changes in insulin binding to receptors of liver plasma membrane were observed after space flight for 7 and 14 days (Macho et al. 1994). However, the insulin binding to plasma membrane from fat cells was markedly increased in rats exposed to space flight for 14 days when compared to controls or to synchronous model group (Fig. 5). Similar results of the studies on insulin receptors were noted in rats exposed to restrain hypokinesia (Macho et al. 1988). These findings indicate that after the space flight the elevated insulin plasma levels do not suppress by the mechanism of down regulation the insulin receptors in liver. In adipocytes the receptors are surprisingly increased which is in agreement with augmentation of lipogenesis in adipose tissue of flight rats. These results demonstrated that insulin receptors in adipocytes and in liver cells of rats respond differently on the exposure to space flight. It is necessary to deter-

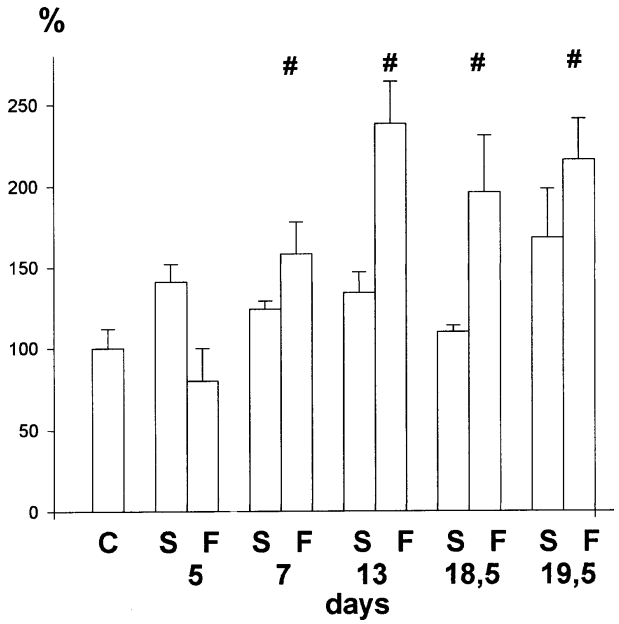

Fig 4 Plasma insulin levels in rats after space flights of various periods. Values in F and S groups (for explanation see Fig.1) are expressed as percentage of controls. Differences are significant # F:C P<0.05

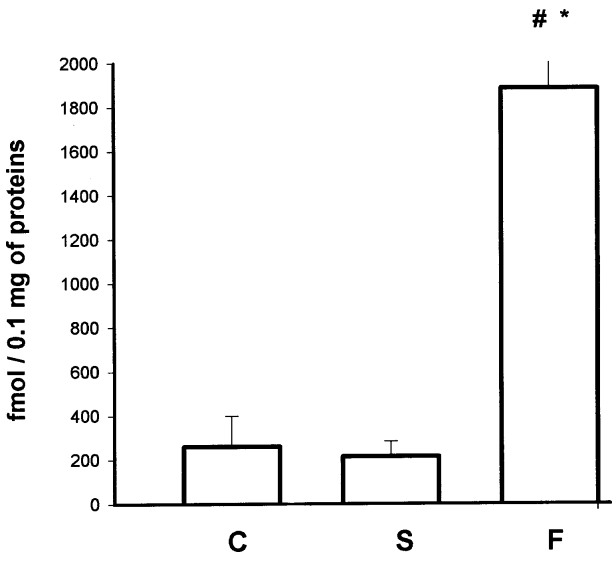

Fig 5 Insulin binding to plasma membrane of adipocytes (B_{max} in fmol/ 0,1 mg of membrane proteins). C – control rats, SM – synchron-model rats and F – rats exposed to space flight. * F:C, S:F P<0.05

mine also the insulin binding in skeletal muscle, because the results on insulin receptors in liver and adipose tissue can not explain the decrease of the rate glucose disappearance during the glucose tolerance tests. The measurement of the content of GLUT-4 transporter in plantaris muscle after 14 days spaceflight did not show any significant changes (Tabata et al. 1998). Because the GLUT-4 is under the effect of insulin it is probable that also the binding of insulin was not affected by space flight. However the other insulin stimulated postreceptor processes in skeletal muscle can be changed by exposure to space flight.

Conclusions

The results of the observations of the exposure of experimental animals to spaceflight showed that endocrine system appears to be sensitive to the conditions of space flight including microgravity. These studies allowed to evaluate morphological changes in the neuroendocrine system and metabolic alterations resulting from changes of hormonal plasma levels. These hormonal changes, however, may be the result of inflight response to microgravity and to the restrained conditions used on board of satellite, or they may be consequences of the stress reactions induced by landing and postflight readaptation to the Earth's gravity (gravitational stress; Popova and Grigoriev 1994). Increased plasma levels of corticosterone and slightly elevated catecholamines in postflight rats are indications of such gravitational stress. These hormones play a role in elevation of plasma glucose and fatty acids, in increased lipolytic activity in adipose tissue, in diminution of lipogenesis and raising of glycogen content in liver (Tigranian et al. 1981; Macho et al. 1982; Macho 1992; Popova and Grigoriev 1994). Such hormonal changes during the spaceflight may be also involved in activating catabolic processes in muscle tissue, in reducing bone formation and microgravity induced osteoporosis, in impairing activity of immune system, in regulation of body fluids and electrolytes and affecting the cardiovascular response to microgravity (Leach et al. 1976; Popova et al. 1993; Hughes 1993; Lesnyak et al.;1996; Fejtek and Wassersug 1999). Currently informations are limited regarding the changes of the content of neuropetides and neurotransmmiters in

brain tissue during the space flight. The role of the changes in their content in altering behavior, diurnal rhythms and working capacity of astronauts must be elucidated.

It is necessary to perform animal experiments during manned space missions or stay on ISS with the collection of blood and tissue samples during the flight for clarification of the specific effects of microgravity and early readapation of animals to Earth gravity. The inflight experiments are important for understanding of hormonal regulatory processes during the space flights. The manned flights show that the plasma cortisol and catecholamine changes are not very high (Leach et al. 1976; Kvetnansky et al. 1983), so as to indicate a potent stimulation of the catabolic processes. The sensitivity of target tissues to hormone has to be also evaluated, in order to identify any changes in the hormone receptors or postreceptor processes, which may in turn increase responses (Henriksen et al. 1986; Tischler et al. 1993; Macho et al. 1994). In such a case even normal hormone concentration could significantly influence growth, differentiation, catabolic or anabolic metabolism in the tissues.

Several hypotheses have been proposed to explain the hormonal changes and their consequences during weightlessness (Leach 1981; Popova et al. 1983; Popova and Grigoriev 1994). However some predicted changes have not occurred in space flights, because besides the microgravity other factors like emotional stress, physical load, altered work/rest cycles and possible drug usage can influence the physiological and endocrine functions. Future animal experiments should focus on the studies of the long-term exposure to microgravity and on inflight responses of animals to various stress loads. The mechanisms of metabolic and functional changes induced by changes of hormonal regulation must be evaluated also on cellular and molecular levels in order to assess the efficacy of various countermeasures applied during the spaceflights. Such studies are important to support the extended stay of human subjects in space environment.

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