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Polyamines, β-Endorphins, Adrenocorticotrophic Hormone, and Prolactin in Prolonged Exposure to Hyperbaric Oxygen

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Vezzani G, Pizzola A, Stefanini P., Rastelli G, Vescovi PP, Casti A, Solari G, Menozzi P. Polyamines, β-endorphins, adrenocorticotrophic hormone, and prolactin in prolonged exposure to hyperbaric oxygen. J Hyperbaric Medicine 1991; 6(3):199–213.—Relations between hyperbaric oxygen (HBO) treatment and endocrine hormone levels are not well known. We investigated the changes in β-endorphin (β-EP), adrenocorticotrophic hormone (ACTH), prolactin (PRL), and polyamine levels during intermittent HBO treatment for 10 days. The measurements were obtained during acute exposure and during chronic exposure. Eight healthy male adults were recruited to undergo 1 HBO treatment per day, for two successive, 30-min periods, for 10 days. A statistically significant increase was observed in values of β-EP and ACTH, each increase respecting normal circadian rhythm. Moreover, PRL levels increased but were not statistically significant during the entire 10 days of treatment. Polyamines (spermine and spermidine) were sampled, and their values increased significantly in the chronic phase of treatment; 3 days after HBO suspension all values returned to base levels.

hyperbaric oxygen, β-endorphins, adrenocorticotrophic hormone, prolactin, polyamines

Introduction

Information regarding the endocrine effects of hyperbaric oxygen (HBO) exposure is poorly documented in current literature. In rats, a functional corticosuprarenal stimulation has been observed (1, 2) and a reduction in testosterone levels (3); in humans, it has been noted that thyroid hormones remain unaffected (4). There is no evidence of a link between HBO and polyamines, molecules that are ubiquitously distributed (5) and are sensitive to a wide range of stimuli (6–8).

The purpose of this paper is to investigate the variations in adrenocorticotrophic hormone (ACTH), β-endorphin (β-EP), and prolactin (PRL) levels and to examine the link between these substances and plasmatic polyamines (spermine and spermidine) in healthy male adults, both in acute and chronic exposure to HBO.

Materials and Methods

Eight healthy male adults belonging to the Federazione Italiana Attività Subacquea (FIAS), aged between 28 and 48 yr, were informed and consented to the study. All observations started at 8:00 a.m., after a 12-h overnight fasting period, with total abstension from alcohol and tobacco as well as maximum reduction of physical activity. Two identical compression exposures were given to each subject, once on compressed air, and another time with O_2. Each compression series was performed for 10 days consecutively, at the rate of one per day, in a multiplace hyperbaric chamber with air compression. The oxygen pressure of 2.8 atm abs was attained on average within 9 min. The HBO exposure time, 60 min in all divided into two periods of 30 min with a 3-min interval in air, was calculated only after the desired oxygen pressure had been attained by oxygen breathing inside the chamber.

The study sequence is detailed at the bottom of Fig. 1. A blood sample was drawn 30 min before each subject entered the chamber (A), at the time of entry (B), at 30 min, and at 60 min after exposure (C) and (D). These values reflect the changes induced by acute exposure on Day 1.

On Day 5, two further blood samples were taken at the beginning (E) and at the end of the treatment (F).

On Day 10, blood was drawn at the beginning (G) and at the end (H) of the treatment. These values are described as chronic (E), (G), or indicative of the acute exposure in chronic phase (F), (H).

(A), (B), (E), and (G) are considered basal values. All values were redetermined 3 days after suspension of HBO, i.e., 13 days after the beginning of the study (I).

Blood samples were maintained on ice in test tubes containing 0.2 ml of EDTA and 0.2 ml of aprotinine, up to the moment of low temperature centrifugation. The plasma obtained was preserved at −70°C until ACTH and β-EP dosage. ACTH and PRL dosages were performed by radioimmunoassay using the Nichols Institute Kit (St. Juan Capistrano, CA); in our laboratory the sensitivity of the method is 15 pg/ml with intra- and extra-assay coefficients of variation (CV) of 6 and 10%, respectively. β-EPs were determined by an immunoradiometric method using a commercial kit (Allegro, Nichols Institute); the sensibility is 10 pg/ml with intra- and extra-assay coefficients of variations of 7 and 10%, respectively. For polyamine dosage, 1 ml of blood was introduced into test tubes to which 1 ml of perchloric acid (6%) was subsequently added; then an elution in *n*-butanol and extraction of the dansyl

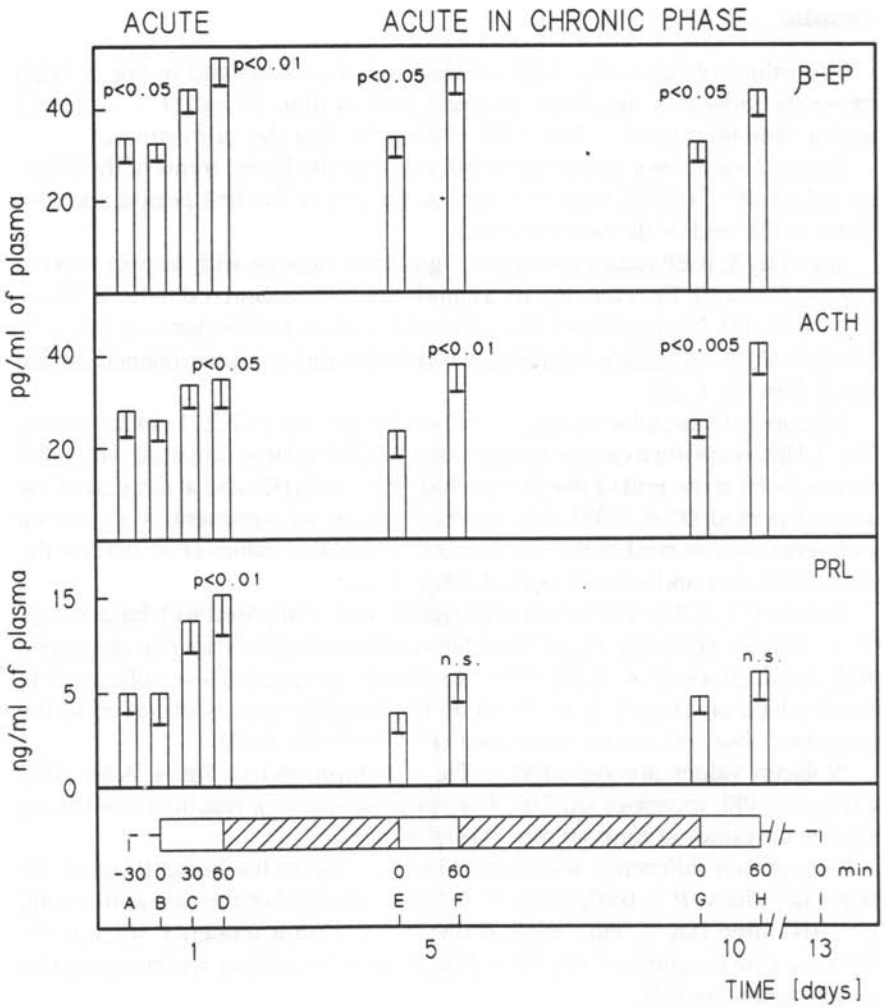

FIG. 1—Plasmatic modifications of ACTH, β-EP, and PRL during acute and acute-in-chronic-phase HBO exposure.

derivates of polyamines by high performance liquid chromatography were performed.

The statistical analysis was carried out by means of variance analysis for repeated values when comparing groups and through the Friedman test when comparing increments.

Moreover, the linear trends of the substances being investigated were taken into consideration after HBO exposure with reference to the values obtained after exposure to air at 2.8 atm abs for 60 min (9).

Results

The values obtained for β-EP are shown in Fig. 1 *top* and in Fig. 2. HBO exposure induces a significant increase, both at time 30 min ($P < 0.05$) (C) and at time 60 min ($P < 0.01$) (D), during the first day of treatment.

Figure 2 *top* shows a significant difference in the linear trend of the HBO-air values ($P < 0.001$), with $P = 0.02$ at the end of the first period and $P < 0.001$ at the end of the second period.

After Day 5, β-EP values undergo a significant increase with respect to basal values ($P < 0.05$; F). After Day 10, a significant rise is observed in basal values ($P < 0.05$; H). No significant variations are seen in basal values on Day 1, 5, 10 (B, E, G). No significant difference is noted in the increase obtained during the 3 days (D, F, H).

Adrenocorticotrophic hormone values are shown in Fig. 1 *center* and in Fig. 3. HBO exposure causes an increase in ACTH values compared with basal values, both at the end of the first period ($P < 0.05$) (C) and at the end of the second period ($P < 0.05$) (D), on the first day of treatment. A significant difference is observed in the linear trend of HBO/air values ($P < 0.02$) at the end of the first and second periods (Fig. 3 *top*).

After Day 5, ACTH levels increased significantly compared with basal values ($P < 0.01$; F). After Day 10, ACTH values undergo a significant rise compared with basal values ($P < 0.005$) (H). No significant variations are observed in basal values on Days 1, 5, or 10 (B, E, G). No difference is observed in the increases obtained on the same days ($P < 0.88$) (D, F, H).

Prolactin values are reported in Fig. 1 *bottom* and in Fig. 4. After HBO exposure, PRL increases on Day 1 at time 30 min (C), reaching statistically significant values at time 60 min (D) ($P < 0.001$).

A significant difference is observed in Fig. 4 *top* in the linear trend of the HBO-air values ($P < 0.003$) with $P < 0.01$ at the end of the first and second periods. After Day 5, PRL levels show an increasing tendency (F) but the increase is not significant (F). After Day 10, there is a rising tendency but this is not significant (H).

Polyamines (Figs. 5 and 6)

During Day 1 of HBO exposure, polyamine levels reveal no significant variations with respect to basal values. The comparison of linear oxygen-air levels reveals no appreciable difference.

After Day 5, basal values increase significantly in comparison to the basal values of Day 1 [$P < 0.02$ for spermine (SPM), $P < 0.01$ for spermidine (SPD)] and do not undergo significant variations at the end of the treatment at time 60 min. After Day 10, basal values increase with respect to those of Days 1 and 5 ($P < 0.007$ for SPM, $P < 0.001$ for SPD) and remain unvaried after 60 min of HBO treatment. On Day 13 (3 days after suspension of HBO), values obtained correspond with basal values at the beginning of the experiment.

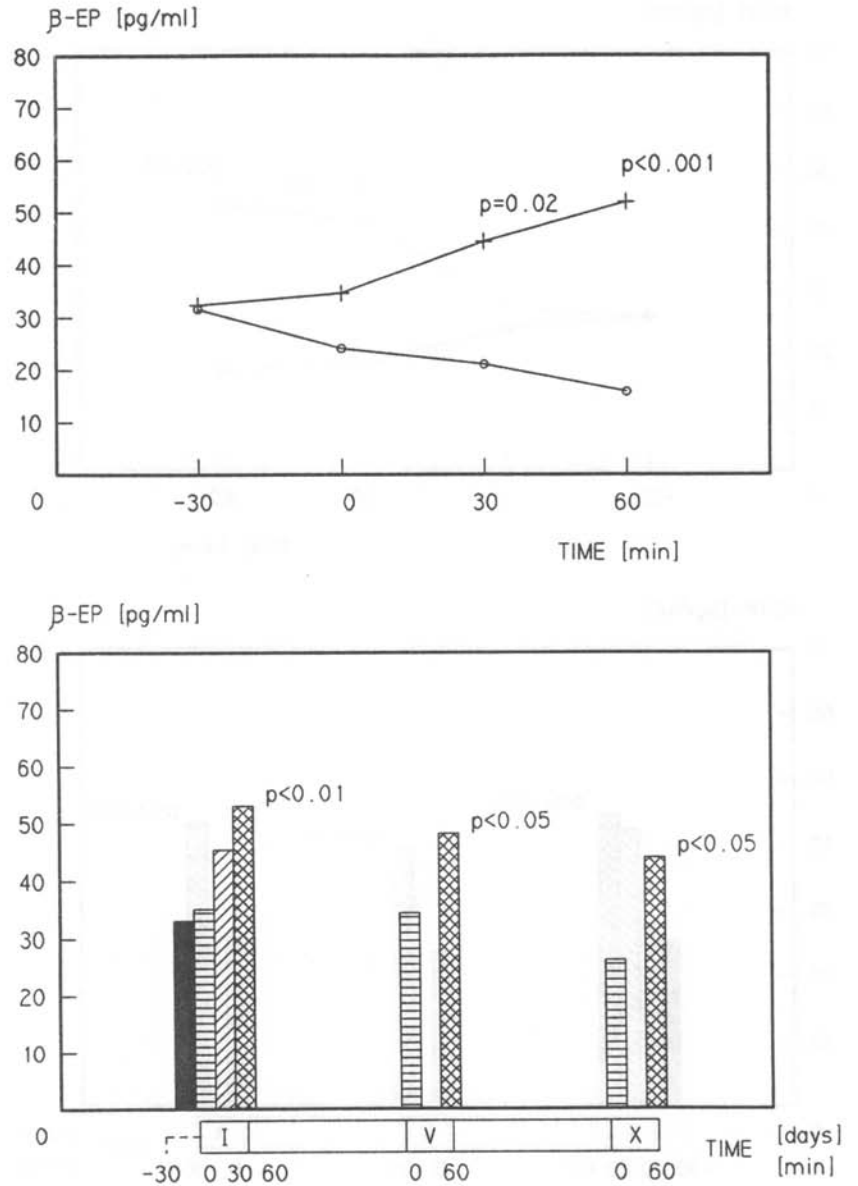

FIG. 2—*Top*, linear trends of β-EP (pg/ml) in acute exposure to compressed air (*open circles*) and to HBO (*plus signs*). *Bottom*, trends in acute HBO exposure (Day 1) and in acute-in-chronic-phase exposure (Days 5–10).

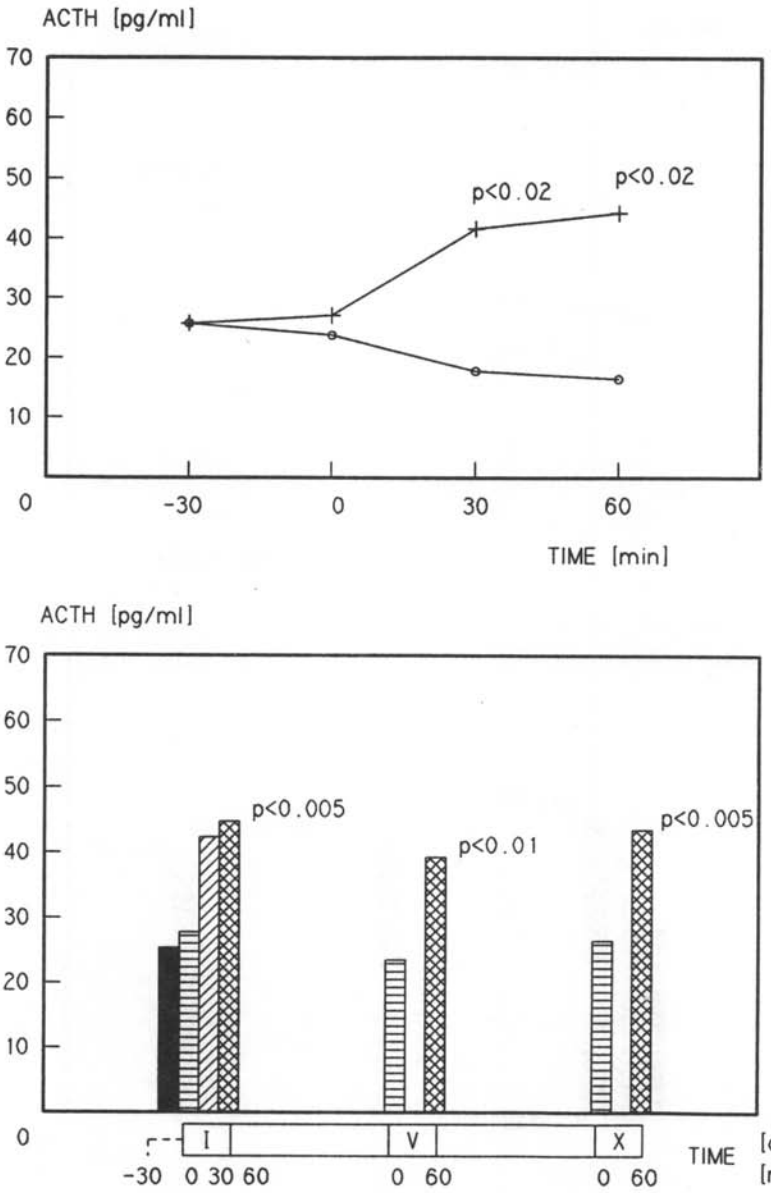

FIG. 3—*Top*, linear trends of ACTH (pg/ml) in acute exposure to compressed air (*open circles*) and to HBO (*plus signs*). *Bottom*, trends in acute HBO exposure (Day 1) and in acute-in-chronic-phase exposure (Days 5–10).

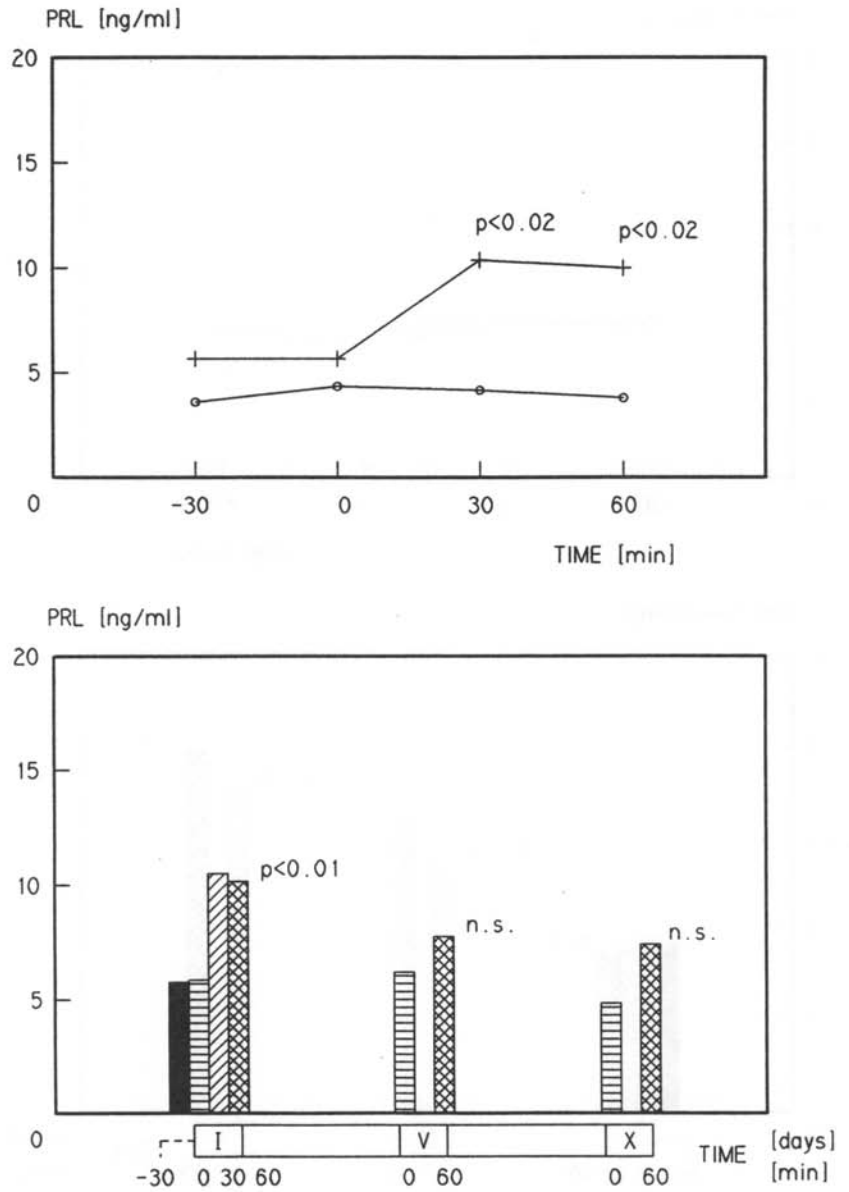

FIG. 4—*Top*, linear trends of PRL (ng/ml) in acute exposure to compressed air (*open circles*) and to HBO (*plus signs*). *Bottom*, trends in acute HBO exposure (Day 1) and in acute-in-chronic-phase exposure (Days 5–10).

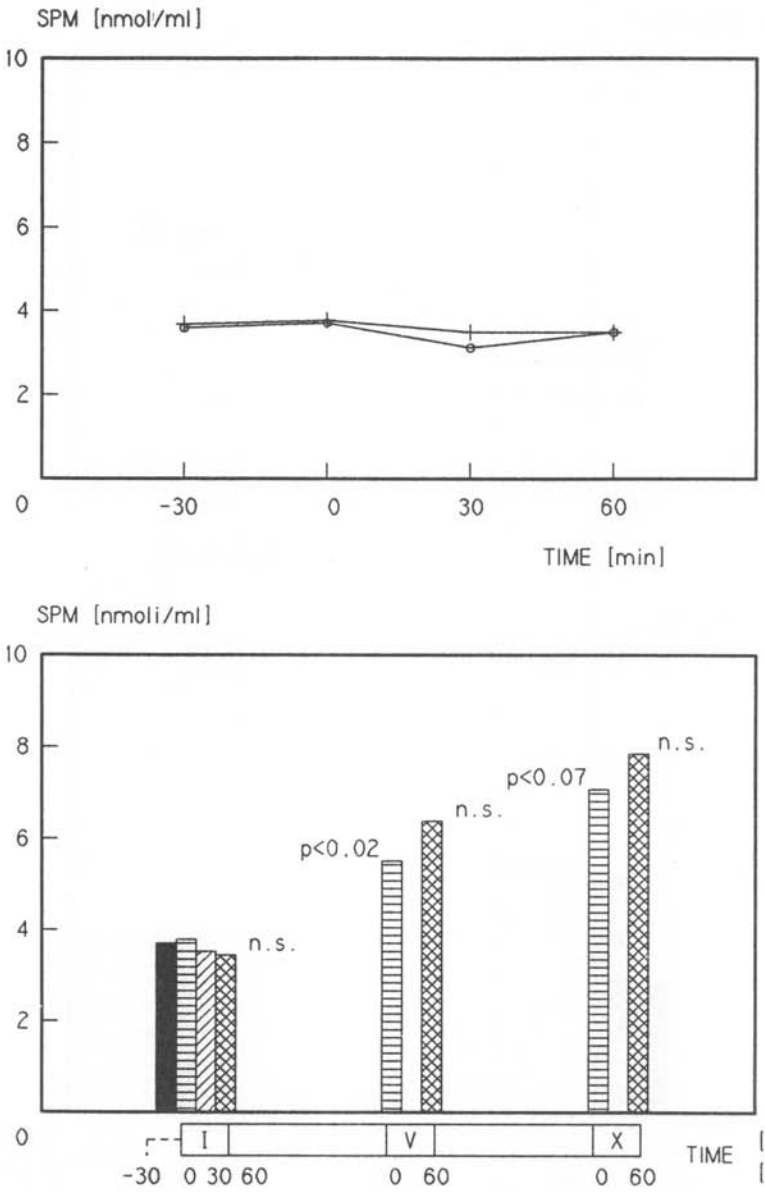

FIG. 5—*Top*, linear trends of spermine (nm/ml) in acute exposure to compressed air (*open circles*) and to HBO (*vertical lines*). *Bottom*, trends in acute HBO exposure and in acute-in-chronic-phase exposure. *Note* the significant increase in the basal values of Days 5 and 10 with respect to those of Day 1 (also for SPD values).

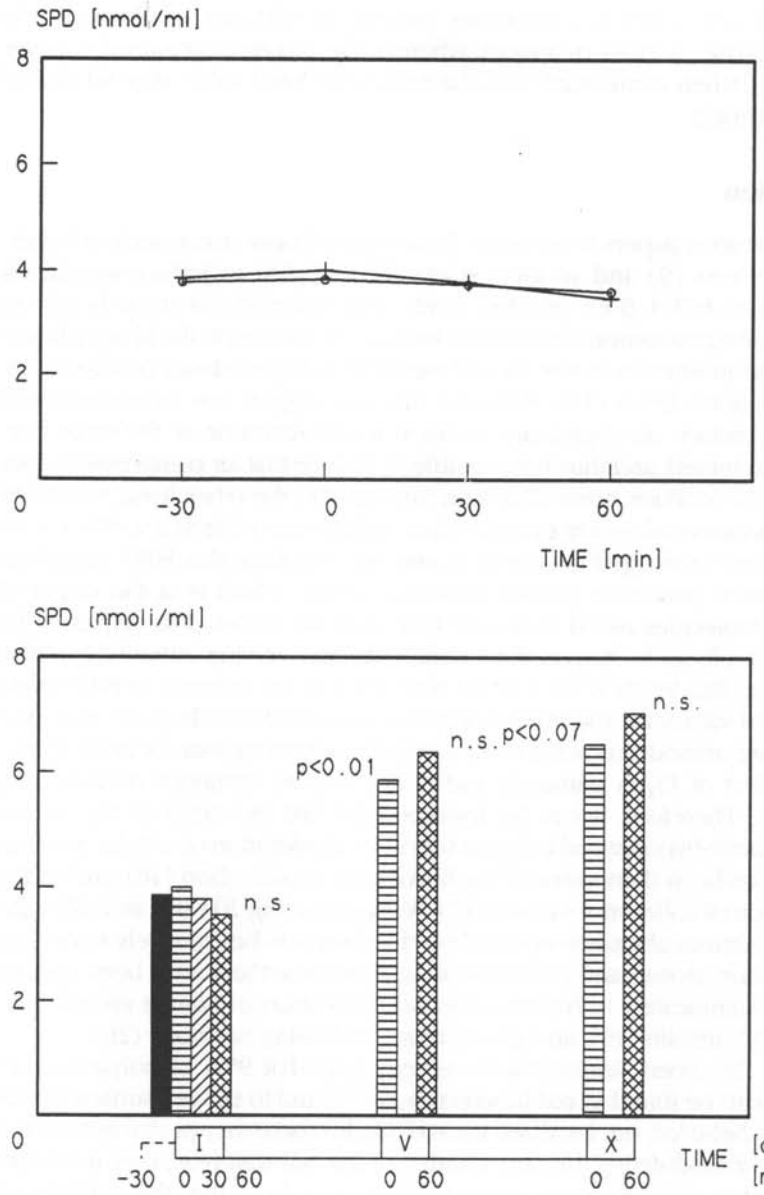

FIG. 6—*Top*, linear trends of SPD (nm/ml) in acute exposure to compressed air (*open circles*) and to HBO (*vertical lines*). *Bottom*, trends in acute HBO exposure and in acute-in-chronic-phase exposure. *Note* that on Days 5 and 10 values at time 60 min are not significant compared with the respective basal values (also for SPD levels).

To conclude, there is a significant increase in SPM and SPD levels in the chronic phase of HBO treatment, whereas the increase measured is never significant when confronted with the respective basal value after 60 min of HBO treatment.

Discussion

In numerous papers it has been demonstrated how stress, such as hyperthermic stress (9) and surgical stress (10), is able to induce significant increases in ACTH, β-EP, and PRL levels. It is believed that stress is able to stimulate the production of serotonin and catecholamines in the hypothalamus (11); these substances in turn induce increases in hypothalamic corticotropin-releasing factor levels (12). However, our data suggest that air compression does not induce any significant variation in the secretion of the three hormones examined, and thus it is possible to deduce that air compression does not in itself produce stress effects on humans. On the other hand, variations in the hormone values are evident when patients are subjected to HBO, both in acute and prolonged treatment. It may be postulated that HBO stimulates the common precursor proopiummelanocortine, which is at the origin of the two molecules ACTH and β-EP (13) that are secreted in sequence by adenohypophysis in response to numerous and varying stimuli (14). The increase in PRL levels is likely to be secondary to the increase in β-EP levels (15). Basal values for the three hormones examined (ACTH, β-EP, and PRL) (Fig. 7) are unmodified at the various checks, suggesting that the acute stimulating effect of O_2 is transitory and is not able to influence normal daily secretion. Therefore, it can be hypothesized that the effect of O_2 on the hypothalamic-hypophyseal cells consists in an acceleration of cellular metabolism, as has been demonstrated for fibroblastic proliferation (16), and in the production of collagenous tissue (17). Polyamines (Fig. 8) (SPM and SPD) are biogenic amines ubiquitously distributed and seem to be intimately associated with cellular growth and cell duplication processes; these have been seen to increase significantly in humans after administration of human growth hormone (18), insulin (19), and gonadotropin-releasing hormone (20).

It has also been demonstrated recently (21) that 95% of polyamines in blood are to be found in red blood cells, not bound to the cell surface as was generally believed, but localized intracellularly where they probably have the function of modulating the permeability of the cell wall (e.g., they modulate the mobility of erythrocyte glycoproteins). It is held that the majority of endocrine and nervous stimuli are able to modify the polyamine concentration in the hypothalamus and adenohypophysis (19).

We have already demonstrated in previous papers (e.g., 9) that air compression at 2.8 atm abs has no effect on the secretion of β-EP, ACTH, or PRL and polyamines, whereas HBO treatment increases SPM and SPD levels, but only in the case of prolonged exposure Fig. 9.

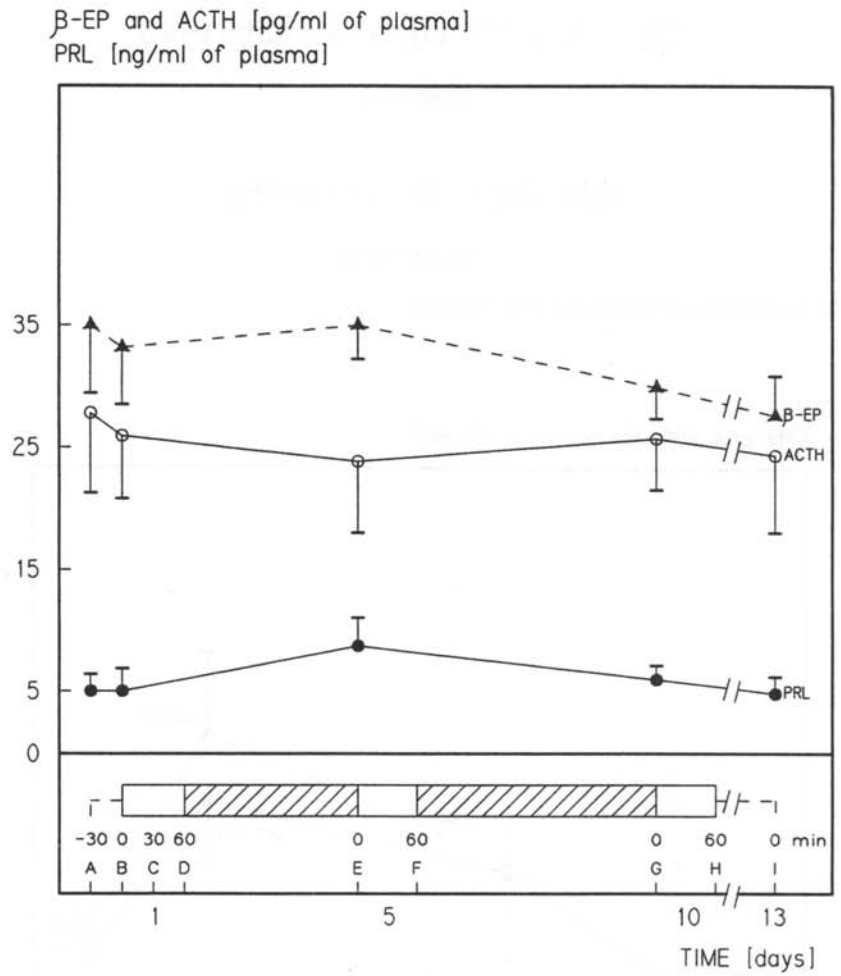

FIG. 7—Effect of HBO treatment on basal plasma concentration of ACTH, β-EP, and PRL on Days 1, 5, 10, and 13 (3 days after HBO suspension).

When compared with the corresponding basal value, the polyamine levels after HBO treatment might be interpreted on the basis of an increase in cellular proliferation and/or of the cellular reaction occurring as a response mechanism to the modifications induced by HBO (22). In both circumstances, stimulation of the genetic apparatus and of protein synthesis, in which polyamines play a fundamental role, is observed. It has been hypothesized that the union of ribosomal sub-units 30 S and 50 S, fundamental in the protein synthesis process, is conditioned by the presence of SPM, SPD, and Ca^{++} ions (23). Moreover, the polyamine-hormone association and the link between

$$H_2N-(CH_2)_3-NH-(CH_2)_4-NH-(CH_2)_3-NH_2$$

Spermine

$$H_2N-(CH_2)_3-NH-(CH_2)_4-NH_2$$

Spermidine

FIG. 8—Structure of SPM and SPD molecules.

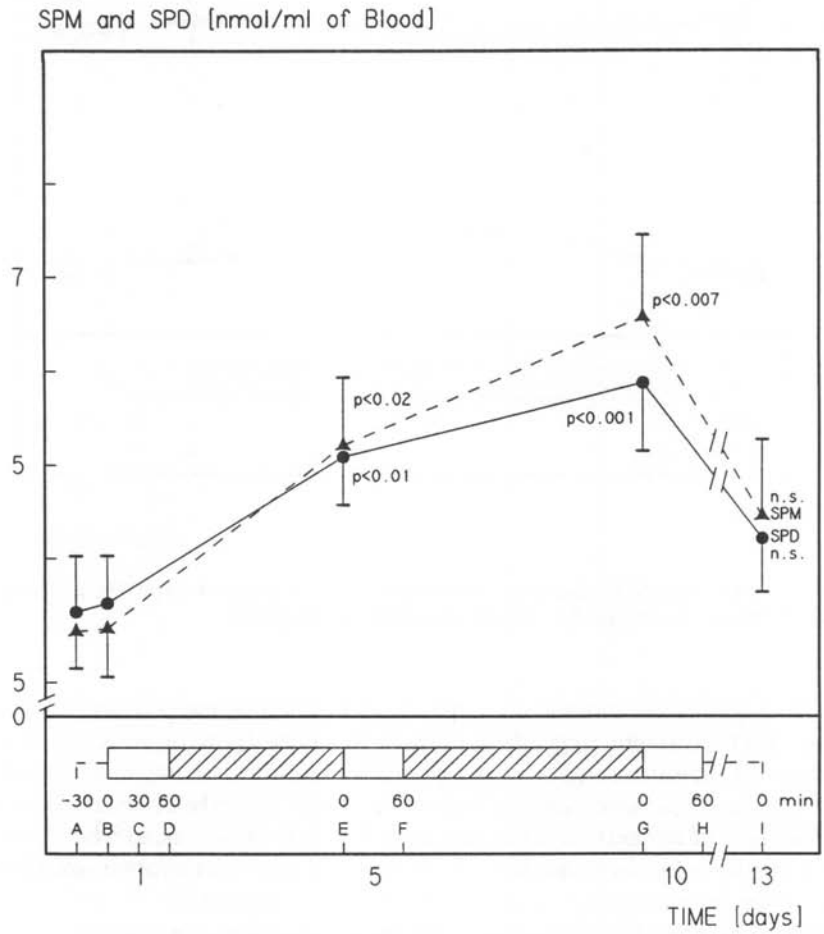

FIG. 9—Spermine and SPD variations (basal values) during prolonged HBO treatment.

polyamines and endocrine stimuli (Fig. 10) have been fully documented (24). Although the mechanism has not been unequivocally clarified in humans, this link, illustrated by an increase in blood-cell associated polyamines after hormonal stimulation, might indicate a cellular response to the event controlled by the hormone or might accompany and stimulate the action of the hormone itself (25, 26).

Although we lack conclusive data, the nonresponse of polyamine to HBO administration in acute phase would lead us to conclude that O_2 needs a latent period before inducing polyamine secretion, which is exactly what happens

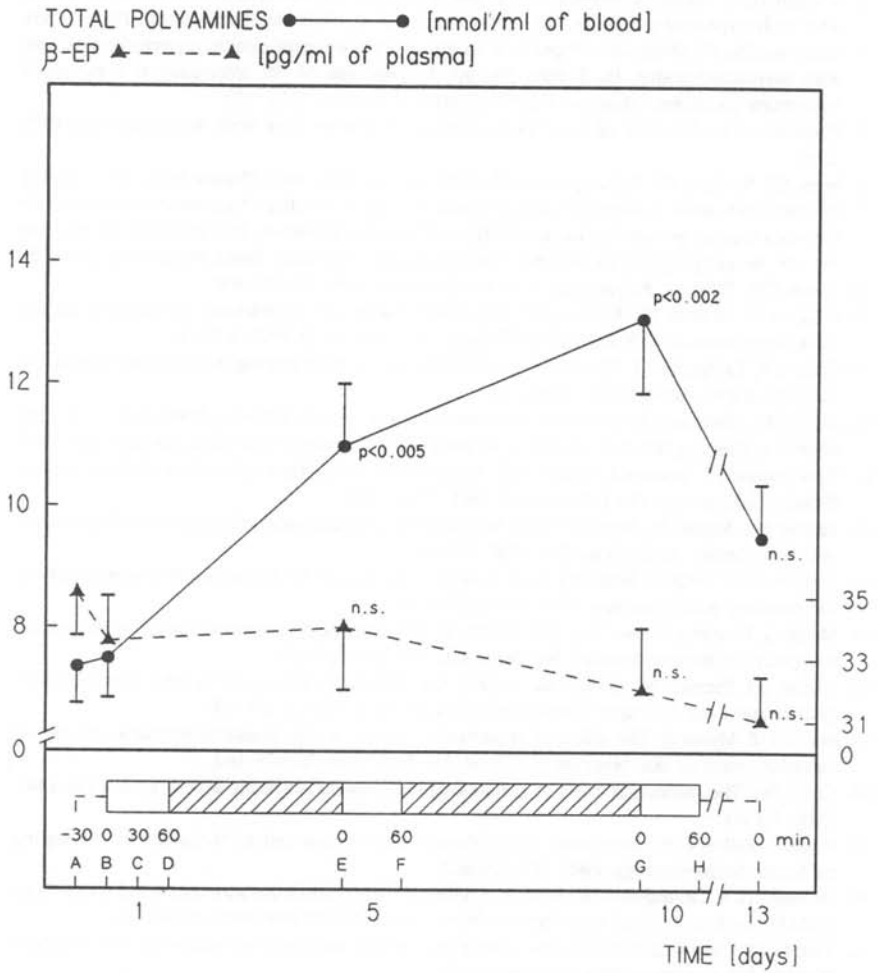

FIG. 10—Total blood polyamine levels and β-EP levels in prolonged HBO treatment. Values registered are basal values.

in the chronic phase. Thus it may be concluded that prolonged HBO treatment can induce a considerable increase in polyamine levels, whereas acute HBO treatment produces an evident neuroendocrine reaction (as has been observed in the secretion of β-EP, ACTH, and PRL).

References

1. Saito H, Ota K, Sageusa T, et al. Increased adrenocortical function in man and rat following hyperbaric oxygen treatment. In: Smith G., ed. Proceedings of 6th international hyperbaric congress. Scotland: University of Aberdeen Press, 1977: 159–163.
2. Golinov PP, Rogatsky GG, Nikolaeva NY. Glucocorticoid receptors in the lungs in hyperbaric oxygenation. Patol Fiziol Eksp Ter 1986; 2:32.
3. Rockert HDE, Haglid K. Reversible changes in the rate of DNA synthesis in the testes of rats after daily exposure to a hyperbaric environment of air. IRCS Med Sci Biochem 1983; 11:531.
4. Rakhmatullin IG, Halley MA. Hyperbaric oxygen in pre- and post-operative periods of patients with thyrotoxic goitre. In: Yefuny SN, ed. Proceedings of 7th international congress of hyperbaric medicine. Moscow: USSR Academy of Science, 1981: 373.
5. Bachrach U. In: Function of naturally occurring polyamines. New York: Academic Press 1973; 1:211.
6. Pegg AE, McCann PP. Polyamine metabolism and function. Am J Physiol 1982; 243:212–221.
7. Bernasconi S, Reali N, Barioglio MR, Orlandini G, Casti A. Further observation on relationship between human growth hormone (hGH) and blood polyamines. In: Chiumello G, Sperling M., eds. Recent progress in pediatric endocrinology. New York: Raven Press, 1983: 297–304.
8. Tabor CW, Tabor A. Polyamines. Ann Rev Biochem 1984; 53:479–490.
9. Vezzani G, Vescovi PP. Effetto dell' esposizione acuta all' iperbarismo in ossigeno ed aria solla secrezione di ACTHe, β-EP nell'uomo. Med Sub ed IP 1991; 6:10–12.
10. Dyama T, Takiguchi M. Plasma levels of ACTH and cortisol during halotane anesthesia and surgery. Anesth Analg 1970; 49:363–367.
11. Jones MT, Hillhouse E, Burden J. Secretion of corticotropin releasing hormone in vitro. In: Martini L, Ganong WF, eds. Frontiers in neuroendocrinology. New York: Raven Press, 1976.
12. Berkenbosch F, Vermes I, Tilders FJH. Epinephrine as a potent releaser of immunoreactive β-endorphin in rats. Eur J Pharmacol 1981; 72:97–103.
13. Eipper BA, Mains RE. Structure and biosynthesis of proadenocorticotropin/endorphin and related peptides. Endocrinol Rev 1980; 1:1–22.
14. Guillemin R, Vergo Y, Rossier J, et al. β-Endorphin and ACTH are secreted concomitantly by the pituitary gland. Science 1977; 197:1367–1368.
15. Meites J, Bruning F, Van Vugt DA, Smith AF. Relation of endogenous opioid peptides and morphine to neuroendocrine functions. Life Sci 1975; 24:1325.
16. Mehm WJ, Pimsler M, Becker RL, Lissner CR. Effect of oxygen on in vitro fibroblast cell proliferation and collagen biosynthesis. J Hyper Med 1988; 3:227–234.
17. Meltzer T, Myers B. The effect of hyperbaric oxygen on the brushing strength and rate of vascularization of skin wounds in the rat. Am Surg 1986; 52:659–662.
18. Conn PM. The molecular basis of gonadotropin-releasing hormone action. Endrocrinol Rev 1986; 7:3–11.
19. Roger L, Fellows RE. Stimulation of ornithine decarboxylase activity by insulin in developing rat brain. Endocrinology 1980; 106:619–625.
20. Bernasconi S, Orlandini G, Reali N, et al. Effect of GnRH administration on blood polyamines and LH levels in normal and obese children. Horm Metab Res 1988; 20:648–651.
21. Tadolini B, Casti A. Intracellular location of polyamines associated to red blood cells. Biochem Biophys Res Commun 1986; 134:1365–1371.
22. Thet LA, Parra SC, Shelburne JD. Repair of oxygen-induced lung injury in adult rats. The role of ornithine decarboxylase and polyamines. Am Rev Respir Dis 1984; 129:174–181.

23. Moruzzi G. Principi di chimica biologica. Libreria Universitaria L. Tinarelli, Bologna, 1973.
24. Janne J, Poso H, Raina A. Polyamines in rapid growth and cancer. Biochim Biophys Acta 1978; 473: 241–293.
25. Koenig H, Goldstone A, Lu CY. Polyamines regulate calcium fluxes in a rapid plasma membrane response. Nature 1983; 305:496–501.
26. Hougaard DM, Larsson LI. Localization and possible function of polyamines in protein and peptide secreting cells. Med Biol 1986; 14:89–94.