SYNTHETIC ACTH ANALOGUE SEMAX DISPLAYS NOOTROPIC-LIKE ACTIVITY IN HUMANS

A.Ya. Kaplan , A.G. Kochetova , V.N. Nezavibathko, T.V. Rjasina , Ashmarin I.P.

Dept. of Human and Animal Physiology, Faculty of Biology, Moscow State University, Moscow 119899, Russia

(Accepted July 30, 1996)

SUMMARY

Nootropic properties of ACTH4-10 analogue, Semax, were demonstrated in experiments with human volunteers. The antihypoxic effect of Semax was shown by EEG analysis after short-term hyperventilation. Semax induced changes in the EEG similar to those seen after administration of typical nootropic drugs. Particularly important was the long-term (20-24 h) beneficial action of Semax on the work efficiency of operators after intranasal administration of 0.25- 1.0 mg of the peptide (about 4.0 - 16.0 /xg/kg body weight).

KEY WORDS: neuropeptides, ACTH analogues, cognitive activity, EEG, memory, attention, hypoxia, ischaemia.

INTRODUCTION

The achievements of neuropeptide research have resulted in the development of modulators of memory processes and other cognitive functions. Among them, the family of corticotrophin (ACTH) fragments and their analogues has attracted particular attention.

There have been many publications about the influence of various ACTH4-7 analogues in humans (1-3, and others). The synthetic peptide Semax (MEHFPGP) is one such analogue (3-11). While its influence on memory and vigilance is more or less similar to that of other analogues, long-term enhancement of mental ability in the field of operator work combined with an antihypoxic action have so far been described for Semax only.

The aim of this paper is to analyse the influence of Semax on a number of central nervous system functions in humans, namely operator activity and quantitative estimation of human EEG during cognitive activity and during transitory cerebral ischaemia induced by hyperventilation.

CCC 0893-6609/96/050115-09

MATERIALS AND METHODS

Determination of the operators' activity parameters.

Recently "computational reactography" methods which permit estimation of parameters of human sensorimotor activity have been used more and more to determine the efficacy of drugs (12-15). Such a computer-based examination of sensorimotor activity takes only 5-20 min.

The operators of Power Plant (Perm, Russia) took part in the experiment. Each operator was tested four times according to the two-shift working regime at Power Plant: at the beginning and the end of an 8-hour watch on the first and second working day.

In the computational examination, 160 blocks of random number images were displayed to the operator, who was asked to memorize them. Forty test numbers were presented after number image presentation in every block. Some of the test numbers were identical to the number images. Operators were instructed to press the computer button "space" as fast as possible when identical test numbers appeared. The results are presented as the number of right responses, the number of wrong responses (on non-identical test numbers), and the number of false responses which occurred spontaneously, without any test number presentation.

Semax was administered by the nasal route at a dose of 0.4 ml (1.0 mg of peptide in physiological solution; about 16.0 /ig/kg body weight). The control group of operators received placebo (physiological solution). The study was carried out in a double blind fashion. Sixteen male operators took part in the full cycle of experiments, eight of whom were selected at random to receive Semax and eight of whom received placebo 60-90 min before the test. The subjects were then given instructions and started the test (15 min duration). The repeated test was held at the same day at the end of working shift. The same subjects took part in the same experiment on the second day of the working cycle. *EEG examination during cognitive activity.*

Eleven healthy young male volunteers (ages 23-27) participated in this study. Subjects reclined comfortably in a special recording room with open eyes in the dark. EEGs were recorded from right occipital area of the brain (02 electrode position according 10/20 system) referred to the linked ears, as is accepted in pharmacoelectroencephalography (18). Seven to eight 1-minute EEG records were obtained for each subject just before and 40 minutes after the intranasal administration of 0.25 mg of Semax (6 subjects) or placebo (5 subjects). The EEG was recorded during the standard test of memorizing visual images (19). In order to study the spectral changes after drugs administration, we calculated relative spectra as the log power density minus the mean log power density in the 1-30 Hz range. Then we assessed the subtraction spectra by using Student's t-test to determine the significance of differences between pre- and postdrug EEG effects (Fig.4.) *EEG examination during posthyperventilation cerebral ischaemia.*

Nine healthy male volunteers (ages 20-40) took part in this study. Each subject was examined three times with 7-8 day intervals in a double-blind, control regime. Baseline physiological functions were monitored simultaneously with EEG registration: ECG, oculogram, breathing frequency, arterial pressure. An EEG from the right occipital region was recorded for 3 minutes before and 1 minute immediately after hyperventilation (3 min). The hyperventilation intensity was kept at a level of 30 respiratory acts in 1 minute with 50-60% increase in heart frequency from starting level.

RESULTS

Semax influence on the operator's activity.

The number of correct responses in the control group receiving placebo was considerably

decreased to 47% (Fig.l.B/A) and the number of incorrect responses was increased to 120% (Fig.2.B/A) at the end of the first working day. In the test group which took Semax 1-1.5 hours before testing the number of correct responses decreased to 91 % (Fig.l.B/A). Moreover, the number of incorrec responses in the test group decreased by 35 % (Fig.2.B/A).

The morning examination on the next day compared with the first morning (Fig.l.C/A) showed that the group treated with Semax made more correct responses (71 %) than the control group tha received placebo (41%). All changes in operator activity were statistically significant at $p<0.01$ (Wilcoxson criterion). It is important to stress that operators did not take drug again until after the morning examination on the second day, and so the significantly higher number of correct responses in the test group may point to the prolonged favourable effect of Semax administered 20 - 24 hours before. The considerable (by 31%) reduction in the number of incorrect responses made by the test group on the morning of the second day further reflects this prolonged beneficial effect of Semax. This parameter was even increased by 20% in the control group treated with placebo (Fig.2.C/A).

The same picture was obtained in the evening of the second working day (Fig. 1,2 D/A) with regard to the correct responses but the number of incorrect responses dramatically increased in the control group (by 50%, Fig.2. D/A) without there being significant changes in the number of incorrects responses in the test (Semax) group.

The number of false (spontaneous) responses had different dynamics (Fig.3.B/A). The number of such responses decreased on the evening of the first day in the control group (by 65 %, $p< 0,010$) more rapidly than in the group that received Semax (by 23%, $p<0,01$). Moreover, this parameter increased slightly (33%, $p<0,01$) at the end of the experiment in the group that received Semax (Fig.3.D/C). Thus, the use of Semax increases the effectiveness and precision of an operator's work. It points to optimization of attention and short-term memory (12). Probably these effects are a manifestation of a more generalized influence of Semax on cognitive processes, an influence which is typical for nootropic drugs (16). We cannot explain the observed increase in the number of false (spontaneous) responses after Semax administration. We assume, that there is some anxiogenic component in the spectrum of Semax's behavioural effects, which manifests itself as an increase in the number of false responses. So Semax may be particulary effective for people with low vigilance and decreased anxiety.

These results show that Semax has a positive effect, which is manifested as a prolonged stimulation of attention and short-term memory parameters in humans. A more detailed interpretation of Semax behavioural effects in humans on psychophysiological adaptation is

possible only after further fundamental study of Semax's central effects . For this reason we investigated the EEG effects of Semax and of other psychotropic drugs.

Semax effects on spectral characteristics of human EEG during cognitive activity.

One of the most effective methods of screening psychotropic drugs for possible therapeutic efficiency is to analyse EEG changes (17,18). This method makes it possible to estimate the main vector of a drug's psychotropic effect on the basis of the results obtained after a single drug administration. In particular, amplification of the alpha-band is considered sufficient to classify a drug as an antiamnestic one (16) and inhibition of the delta-band is considered evidence of nootropic activity (17). The aim of this study was to determine the spectral reorganization of the human EEG after the intranasal administration of Semax. The relative power spectra of EEG signal in six standard frequency bands, delta-, theta-, alphal, alpha2, betal and beta2, was calculated on the basis of Fast Fourier Transform algorithm, using a Parzen window for smoothing the spectra. Two-second epochs with 128Hz digitizing were analyzed. The obtained spectra were averaged separately before (spectrum 1) and after (spectrum 2) Semax administration. To determine Semax's effects, a differential spectrum was calculated by subtraction of spectrum 1 values from spectrum 2 values. The subtraction spectra of Semax and placebo are shown in Student criterion units averaged for each subject's group. The values of Student criterion for the differential spectra did not achieve threshold quantities in any frequency band after placebo. Only in the delta band was there a marked tendency to an increase in these rhythms. After intranasal Semax administration the power of the delta band decreased ($p<0,01$) while the power of both alpha rhythm bands ($p<0,01$ and $P< 0,001$) and slow wave band of beta rhythm ($p<0,01$) sharply increased.There was no similarity between the EEG changes after Semax administration and the EEG changes after administration of analeptics and neuroleptics. The EEG changes are in agreement with Semax's antiamnestic potential, and the inhibition of delta rhythm demonstrates Semax's similarity with nootropic drugs (17).

Semax's influence on EEG dynamics during transitory posthyperventilation cerebral ischaemia.

Semax's action in experimental animals and humans encouraged us to further examine this drug's influence on parameters distinctive for nootropic effects. A way to evaluate the effects of nootropics is to estimate the brain's supply of oxygen (20). So it was important to study the central effects of Semax in humans during cerebral ischaemia. One of the most useful models of transient ischaemia of the brain in humans is so-called acute hyperventilation (21 et al). The

ischaemic effects of hyperventilation (HV) are revealed in the EEG as an increase in slow oscillations and an amplitude reduction in the alpha band (22).

Intranasal administration of Semax did not cause considerable changes in the EEG parameters, heart rate, breathing frequency, oculogram and arterial pressure. However, Semax had a significant influence, as revealed in EEG, after HV (table 1). In contrast with EEG before HV, on the first minute after HV and placebo delta and theta rhythms were increased ($p<0,05$) (table 1). However, when Semax was intranasally administered 40-50 min before HV, the above EEG changes were significantly attenuated. On average, the influence of HV on EEG spectral components was decreased to 50-80% (table 1), and totally disappeared in several subjects (4 of 9).

Although we did not estimate absolute EEG effects, these data support again the hypothesis about Semax's nootropic properties. Because typical post hyperventilation EEG changes are detected in clinical cases of acute cerebral ischaemia of different genesis (21), there is a reason to carry out corresponding clinical investigations of Semax's action in the future.

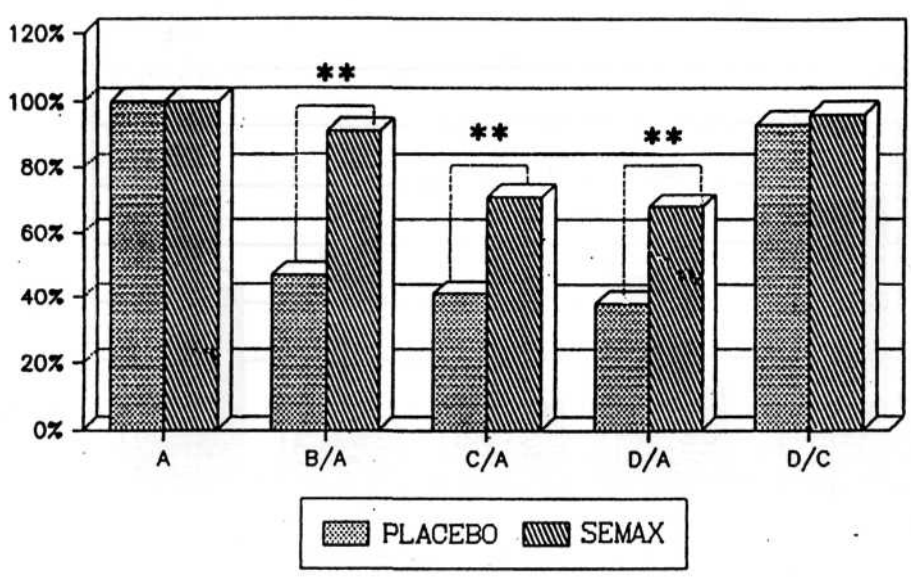

Fig.l. The correct responses to numerical images. Intranasal administration of Semax (1 mg, about 16 /ig/kg of body weight) or placebo 60-90 min before each test. 100 % - average number of correct responses on the 1st day morning. A - 1st working day morning, B - 1st working day evening, C - 2nd working day morning, D - 2nd working day evening. * -$p < 0.05$, * * -$p < 0,001$.

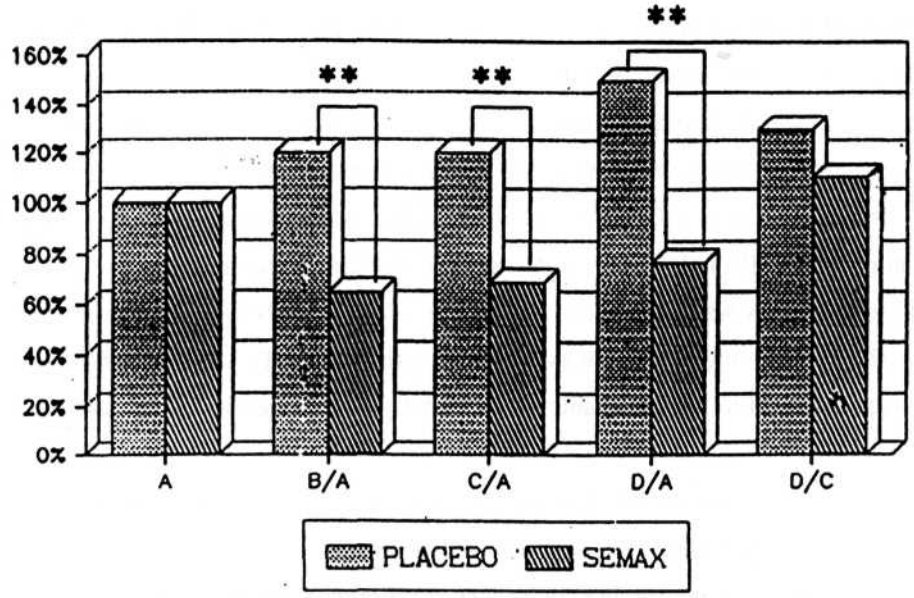

Fig.2. The incorrect responses to numerical images. Intranasal administration or Semax (1 mg, about 16 /xg/kg of body weight) or placebo 60-90 min before each test. 100 % - average number of correct responses on the 1st day morning. A - 1st working day morning, B - 1st working day evening, C - 2nd working day morning, D - 2nd working day evening. * -p < 0. 05, ** -p < 0,001.

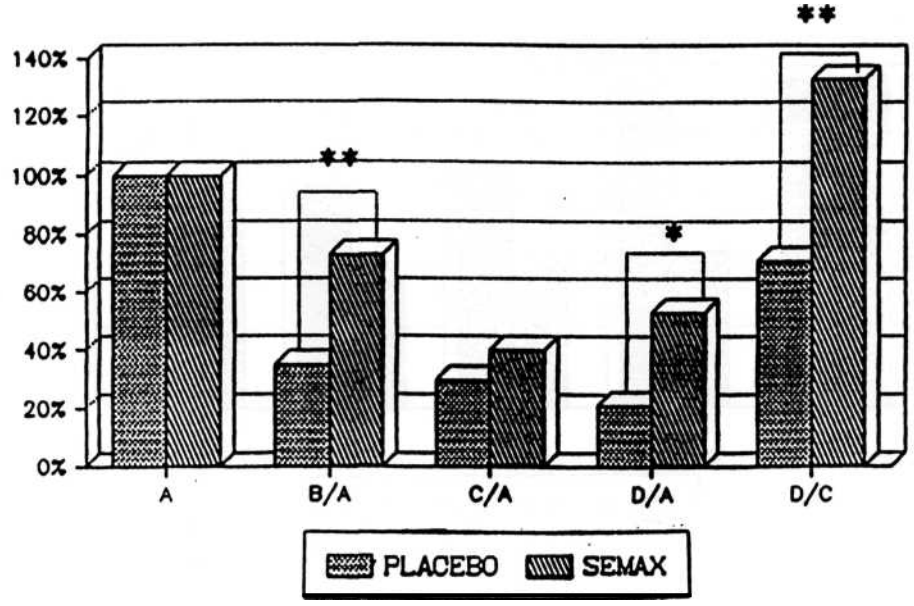

Fig.3. False responses to numerical images. Intranasal administration of Semax (1 mg, about 16 /tg/kg of body weight) or placebo 60-90 min before each test. 100 % - average number of correct responses on the 1st day morning. A - 1st working day morning, B - 1st working day evening, C - 2nd working day morning, D - 2nd working day evening. * -p < 0. 05, * * - p < 0,001.

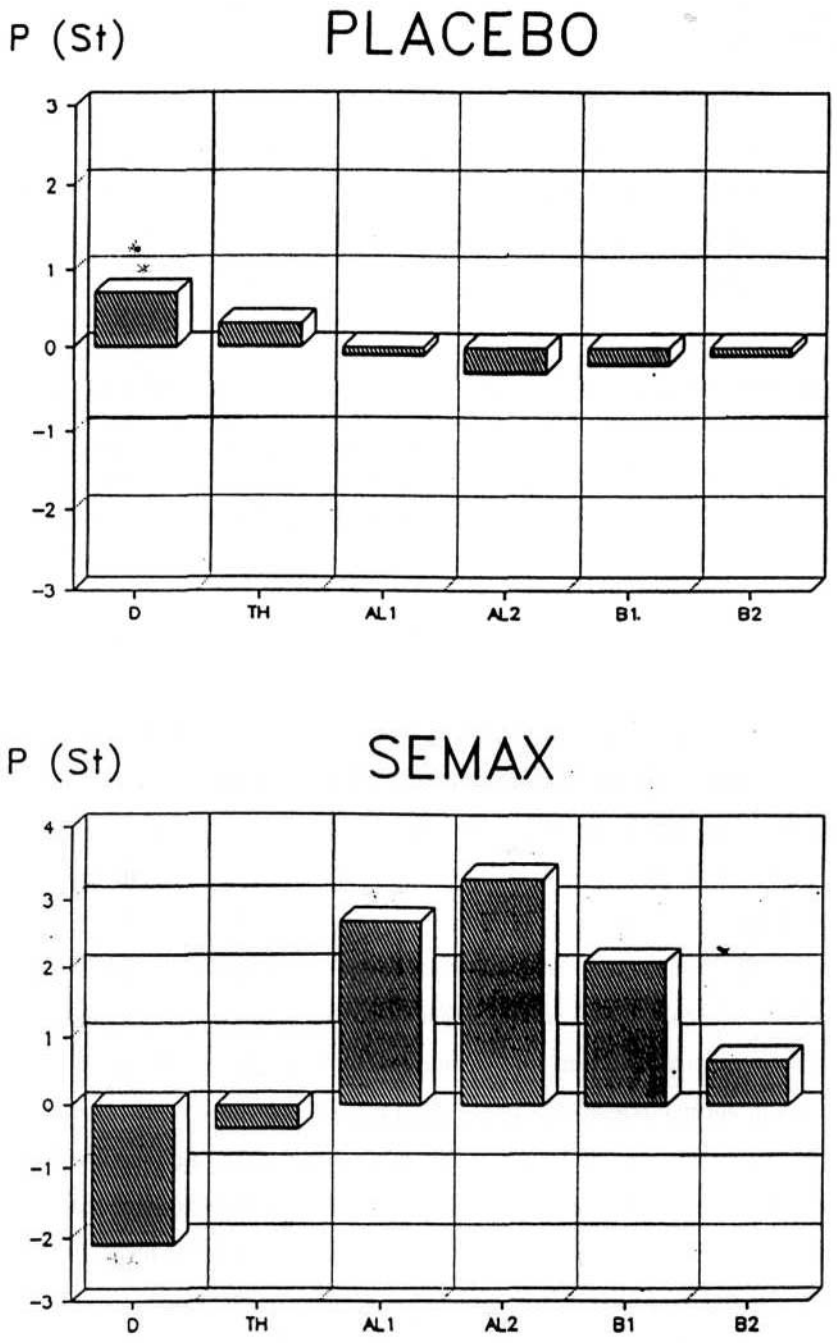

Fig.4. Subtraction EEG power spectrum for the effects of placebo and Semax. Vertical axis: significant differences (Student's t-test) between pre- and postdrug power spectra (1 - $p < 0.05$; 2 - $p < 0.01$; 3 - $p < 0.001$); horizontal axis: different frequency bands of EEG (D - delta, Th -1 heta, All - alphal, A12 - alpha2, Bl - betal, B2 - beta2).

Table 1. Changes in relative spectral power (RSP) of different EEG frequency bands after 3 min of hyperventilation following placebo or Semax administration.

	D	Th	Al	A2	Bl	B2
PL	120.3(5.1)	126.1(4.3)	84.0(3.2)	80.4(3.1)	94.7(6.2)	120.0(5.7)
SM	107.8(4.3)	105.4(6.1)	91.8(5.4)	95.6(3.6)	97.2(5.3)	96.9(4.8)
ES	-61.6%*	-79.3%**	+48.6%*	+77.6%**		

D,Th, A1,A2, Bl, B2 - EEG frequency bands; PL - RSP of EEG in the first minute after placebo administration and hyperventilation (100% is the level of RSP before HV); SM - the same, but after Semax administration; ES - the degree of EEG changes correction by Semax after hyperventilation in % of complete effect after placebo administration: ES= (PL-100)/(PL-SM)* 100. Only statistically significant (according to Wilcoxson criterion) changes of ES were shown ("*" -$p<0,05$, "**" -$p<0,001$).

DISCUSSION

The present data show several characteristics of Semax which are important for fundamental studies and for the clinical use of the drug. Semax could be used to achieve a long-term increase in operator work efficiency. The increase on attention, short-term memory, and in the ability to make the correct decision, which were observed during the day after a single Semax administration, appear to be attractive characteristics of this drug. At the same time, some increase in the anxiety level may be useful for improving the vigilance of "relaxed" operators, but could also be a possible source of too rapid solutions. So further investigations in this direction are necessary. It is interesting that Semax has effects other than its influence on cognitive activity. We found that there is a similarity between Semax and nootropic drugs not only in memory stimulation properties, but also in antihypoxic effects in the central nervous system. This is an additional reason to consider Semax for possible clinical use for the treatment of different hypoxic states. There is an obvious question, namely what is the main and initial Semax effect: direct influence on cognitive and memory processes at a neuronal level or an influence at the level of oxygen supply to the brain. We hope that our further investigations will clarify this issue.

The authors would like to thank Drs. N.V. Korotkova, Ju.K.Malachov, N.V. Ponomareva and V.F. Fokin for their help in organization of the experiments with operators.

REFERENCES

1. Huston, J.P., Hasenohrl, R.U. (1995) Behav. Brain Res. 66, 117.
2. Banks, W.A., Kastin, A.J.(1993) Rev. Neurosci. 4, 365.
3. Hock, F.J., (1995) Behav. Brain Res. 66, 143.
4. Ashmarin, I.P. (1984) Voprosy Meditzinskoi Himii 3, 2.
5. Ashmarin, I.P., Nezavibatko, V.N., Levitskaya, N.G., Koshelev, V.B., Kamensky, A.A.(1995) Neurosci. Res. Commun. 16, 2, 105.
6. Branconier, R.J. (1986) In: International Encyclopedia Pharmacologies and Therapeutics. Section 117: neuropeptides and behavior. Vol. 1: CNS effects of ACTG, MSH and Opioid Peptides, D. de Wied, W.H. Gispen and T.B. van Wimersma-Greidanus (eds). Pergamon, Oxford, p. 421.
7. Kragh-Sorensen, P., Olsen, R.B., Lund, S., Riezen, H.V., Steffensen, K. (1986) Prog. Neuro-Psychopharm. Biol. Psychiatry 10, 479.
8. Elia, D., Frederiksen, S.H. (1984) In: Psychoneuroendocrine dysfunctions. Plenum.medical Book Co., New York.
9. Rockstron, B., Elbert, T., Lutzenberger, W., Birbanmer, N., Fehm, H.L., Voigt, K.H. (1981) Psychoneuroendocrinology 6, 4, 301.
10. Born, J., Kern, W., Pietrowsky, R.I. (1989) Psychopharmacol. 99, 439.
11. Sandman, C.A., Kastin, A.J. (1986) Hormonal Proteins and Peptides 13, 147.
12. Zinchenko, V.P., Velichkovski, B.M., Vuchetich, G.G. (1980) Functional structure of the visual memory.
13. Kaplan, A.Ya. (1989) In: Problems of the design and apply for psychophysiological investigations, M. Nauka, p .25.
14. Leonova A.B. (1984) Psychodiagnostics of the human functional states.
16. Hock, F.J. (1987) Neuropsychobiology 17, 145.
17. Kaplan, A.Ya.(1989) In: Physiology of the peptides, L. Nauka, p. 86.
18. Herrmann, W.M. (1982) In: EEG and Drug Research, N.Y., p. 244.
19. Kaplan, A.Ya. (1991) In: Meditzinskay tehnika. M. Medicina 3, 40.
20. Voronina, T.A. (1989) In: Farmakologicheskaya korrectziya gipoxicheskih sostoyaniy, M. Medicina 125.
21. Kennealy, J.A.,Penovich, P.E., Moore-Nease, S.E. (1986) Electroenceph. Clin. Neurophysiol. 63, 98.
22. Kraaier, V., Van Huffelen, A.C., Wieneke, G.H. (1988) Electroenceph. Clin. Neurophysiol. 70, 377.