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Action of 1,6-Dimethyl-8β-(Bromoisonicotinoyl-Oxymethyl)-10α--Methoxyergoline (Sermion^R) on Some Molecular Processes in the Brain

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The influence of Sermion^R on cellular protein synthesis in the mouse brain was studied in vivo, after an intraperitoneal administration of 50 microgram doses. The brain samples analyzed at different intervals after drug treatment showed a short-term increase of ^{3}H-Leucine incorporation into proteins, followed by a gradual decline of uptake. The latter reached control levels about 18 h after drug administration. Under similar experimental treatments Sermion had no effect on the synthesis of RNA catalyzed by Mg^{++}--ions; however, a slight stimulation of the Mn^{++}-activated RNA synthesis was obserwed.

Mice receiving i. p. injections of 50 micrograms of Sermion showed an increased brain methyltransferase activity; the maximum stimulation was observed one hour after drug treatment. In another set of experiments the capacity of mouse brain preparations to catalyze the methyl group incorporation into the exogenously added transfer RNA (tRNA) was found to decrease with increasing age of the animals; the addition of 10^{-4} M Sermion to the incubation medium enhanced endogenous methylation, especially in the older animals. On the other hand the capacity to methylate the exogenously added heterologous tRNA was not effected by Sermion, irrespective of the age of the animals.

In an attempt to understand the physiological relevance of the biochemical effects of Sermion, we have carried out some studies on the effect of this drug on conditioned learning in rats. The preliminary data indicate that the drug is able to enhance the learning quotients, however, more experiments are in progress to reach a definitive conclusion.

INTRODUCTION

Observable changes in brain function often reflect disturbances of biosynthetic interrelationships between proteins and nucleic acids in brain cells[1]. It is well known, for inctance, that the build-up and degradation of these macromolecules depend on an unimpaired cellular nutrient and oxygen supply; and brain functions are particularly sensitive to drugs affecting this supply[2]. For this reason we have investigated the effects of Sermion (Figure 1), a centrally acting drug, on the cellular metabolism of proteins and nucleic acids in the

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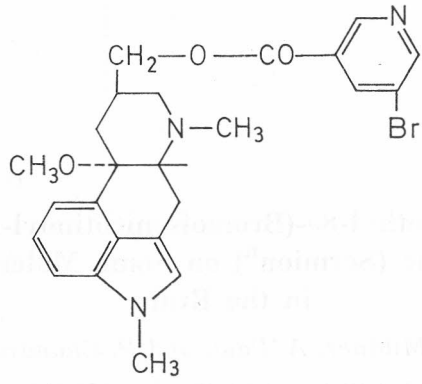

Figure 1. Chemical structure of 1,6-Dimethyl-8β-(Bromoisonicotinoyl-Oxymethyl)-10α-Methoxy-ergoline (SERMION®).

brain, since this drug also possesses manifest vasoactive properties[3,4] and might be capable of modifying the supply processes.

EXPERIMENTAL

Materials and Methods

L-Leucine-^{3}H (sp. act. 5 mCi/mmol), ATP-^{3}H (25 mCi/mmol), S-adenosyl-L-Methionine-^{3}H-Methyl (7.2 mCi/mmol), Protosol, PPO (2,5-diphenyloxazole) and POPOP (2,2-*p*-phenylene-bis-(5-phenyl-oxazole)) were obtained from the NEN-Chemical Company, Dreieichenhain, Germany. Unlabelled substrates (UTP, ATP, CTP and GTP) were supplied by the Nutritional Biochem. Corp., Cleveland Ohio, U.S.A. All fine chemicals (p. a. -grade) were products of Merk AG, Darmstadt, Germany.

Albino mice, weighing about 20 g, were used in our studies. The animals were killed by decapitation and the heads were placed in dry ice until further manipulation. The brains were removed, pooled (2 brains), and homogenized in an all-glass hand--driven homogenizer, using buffer I (0.01 M Tris, 0.0015 M $MgCl_2$, 0.01 M KCl, pH 7.4). Crude particles were removed by passing the homogenate through nylon tissue and the filtrate was collected in a precooled centrifuge tube. The suspension was centrifuged at 4 °C in a Sorvall RC II at 700 × g for 20 min. The resulting sediment was saved for the purification of nuclei, whereas the supernatant was used directly for protein synthesis. The sediment was homogenized in 10 volumes of 0.01 M Tris--buffer, pH 7.4, containing 2.3 M sucrose and 3.3 mM $CaCl_2$, and centrifuged at 40,000 × g for 1 h. The nuclear pellet was then suspended in 0.01 M Tris-buffer, pH 7.4, containing 0.34 M sucrose (1 g tissue per ml) and used immediately for the in vitro RNA synthesis.

Protein synthesis[5] was carried out by incubating 0.3 ml portions of the 700 × g supernatant with 2 μCi of ^{3}H-leucine in 0.3 ml of buffer II (75 mM KCl, 10 mM $MgCl_2$, 15 mM $NaHCO_3$, 15 mM glucose, 20 mM nicotinamide, 2.5 mM NAD, 2 mM ATP; pH 7.4). After incubation for 30 min at 37 °C the reaction was terminated by adding 3 ml of 10% trichloroacetic acid (TCA). The resulting precipitate was centrifuged, washed three times with 5% TCA, and dissolved in 1 ml of protosol. The solution was mixed with 10 ml of toluene scintillator (4 g PPO, 100 mg POPOP, dissolved in 1 liter of toluene) and the radioactivity was counted in a liquid scintillation counter (Nuclear Chicago, Model Mark II). Protein was estimated by the method of Lowry et al.[6]

The RNA-polymerase activity of the purified nuclei was assayed in the presence of Mg^{++}- or Mn^{++}-ions[7,8]. The reaction mixture of the Mg^{++}-activated polymerase was adjusted to a final volume of 0.65 ml and contained: 0.15 ml of nuclear suspension, 50 μmol TrisHCl (pH 8.2), 5 μmol $MgCl_2$, 1 μmol of ATP (2 μCi) and 0.5 μmol (each) of CTP, UTP and GTP. The reaction mixture for $Mn^{++}/(NH_4)_2SO_4$-activated polymerase was identical with the Mg^{++}-containing assay medium, except that 2.2 μmol of $MnCl_2$, and the pH of the system was 7.5. After equilibration at 37 °C the reaction was started by adding 0.15 ml of the nuclear suspension. The reaction mixture was

incubated for 20 min and terminated by the addition of 5 ml of cold 10% TCA. The resulting precipitate was centrifuged, washed with 5% TCA three times and dissolved in 1 ml of protosol. The protosol solution was mixed with 10 ml of toluene scintillation fluid, and the radioactivity was counted. DNA was estimated by the method of Burton[9].

tRNA-methylation in-vitro[10] was carried out with the microsome-free, 150,000 × × g -supernatant (S-150) of mouse brain. This fraction was prepared in the following way: freshly removed mouse brain was homogenized with a four-fold vol. of buffer III (0.25 M sucrose, 10 mM $MgCl_2$ and 1 mM mercaptoethanol in 0.05 M Tris-HCl, pH 7.8). After 2 h centrifugation at 150,000 × g, the supernatant (S-150) was dialyzed overnight against two changes of 1 liter each 10 mM Tris pH 7.8, 1 mM EDTA, 1 mM mercaptoethanol in glycerol-H_2O (1 : 1) at 0—4 °C. The dialyzed material was stored at — 20 to — 30 °C.

The methylating system for exogenous methylation was adjusted to a final volume of 0.22 ml and contained: 170 pmol or *E. coli*- tRNA (E_{260} = 0.1), 20.0 μM Tris-HCl pH 7.8, 2.0 μM reduced glutathione, 2.0 μM $MgCl_2$, 0.1 μCi S-adenosyl-L--methionine-^{3}H-methyl, 0.05 ml methylase fraction (0.2 mg protein), and distilled water to make up a total volume of 0.22 ml. After incubation for 90 min at 35 °C the reaction was stopped by the addition of 5 ml of 10% TCA. The precipitate was collected on a Sartorius membrane filter (diam. 25 mm, pore size 0.45 μm) and washed three times with ice-cold 5% TCA. The filters were air dried and counted in 10 ml of the toluene scintillation fluid.

The system for endogenous methylation was the same, with the exception that no *E. coli* tRNA was added to the reaction mixture.

RESULTS AND DISCUSSION

Quantitative changes in the synthesis of macromolecules in the brain under various physiological states, such as electric shock, druginduced convulsions and exposure to light have been observed by several investigators[11–15], (for review see ref 1). These studies were substantiated by the fact that several other factors like age[1] and hypophyseal hormones[16] also play and important role in the modulation of brain protein synthesis. In recent years, the RNA and protein synthesizing systems of the brain have been used as »targets« to develop encephalotropic drugs such as pyritinol, GABA, magnesium pemoline and others[2].

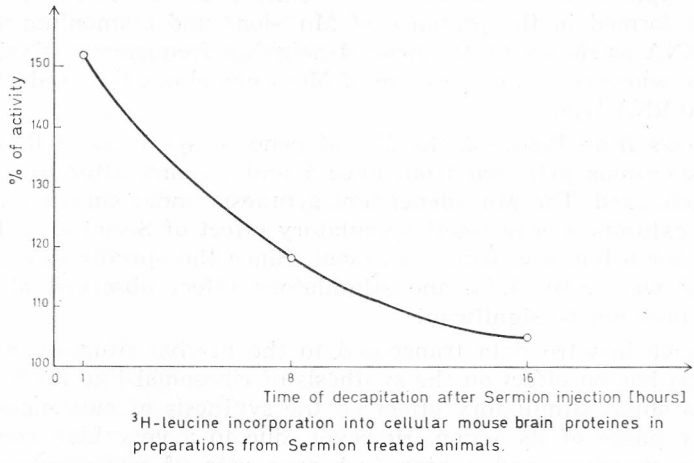

Figure 2. In vivo effect of Sermion on the ^{3}H-Leucine incorporation into cellualar mouse brain proteins. The drug was administered intraperitoneally, and the animals were decapitated at various posttreatment periods, as indicated (Abscissa). The procedure is described under Experimental: 100% value designates an incorporation of 2340 c.p.m./mg protein, under the given experimental conditions. Each value is a arithmetic mean of 4—5 different experiments.

The kinetics of ^{3}H-leucine incorporation into proteins synthesized by 700 x g supernatant of brains from differently treated animals is shown in Figure 2. In these experiments, albino mice were divided into three experimental groups of 10 each. Each of these experimental groups had its own controls with the same number of animals. The experimental groups received 50 μg of Sermion per mouse, dissolved in 0.01 M tartaric acid solution alone. The drug and the solvent were administered intraperitoneally. One, eight and sixteen hours after drug administration the animals in the three experimental groups and their corresponding controls were sacrificed. The brains were removed, frozen in dry ice, and their capacity to synthesize proteins was measured as described under Materials and Methods. Each value is the arithmetic mean of 4—5 different experiments. As shown in Figure 2, there is a significant stimulation of ^{3}H-leucine incorporation into cellular mouse brain proteins in sermion--treated animals. The stimulatory effect of Sermion is, however, time limited. Thus, a maximum stimulation of protein synthesis was seen in the first hour of the drug administration which gradually declined with time. In these experiments the stimulatory response of Sermion decreased almost to the control level 16 h after drug treatment.

The time course of sermion action on brain protein synthesis seems to be related to the metabolic degradation of the drug. The pharmacokinetic studies with ^{14}C-labelled Sermion administered intraperitoneally to rats have shown that more than 98% of the administered drug is excreted in the first 15 hours[17]. Even though the time factor in different studies cannot be compared, one cannot rule out the possibility that the metabolic fate of the drug may contribute to the time course of sermion action on protein synthesis as shown here.

The effect of the drug on RNA biosynthesis under similar experimental treatments is shown in Figure 3. In these experiments, the capacity of a nuclear suspension to synthesize RNA was studied in two different systems. One of these systems contained Mg^{++}-ions, whereas, the other system utilized Mn^{++}-ions supplemented with ammonium sulfate. It has been reported[7,8] that the product formed in the presence of Mn^{++}-ions and ammonium sulfate is a DNA-like RNA as shown by the nearest-neighbor frequency analysis and base composition, whereas, in the presence of Mg^{++}-ions alone, the product formed is of ribosomal RNA type.

As follows from Figure 3, the Mg^{++}-dependent synthesis of RNA by brain nuclear suspensions prepared from mice 1 and 8 hours after drug treatment remains unchanged. The Mn^{++}-dependent synthesis under similar experimental treatments exhibits a very small stimulatory effect of Sermion at 1 hour, but no effect after 8 hours of drug treatment. Since the specific deviation in the experiments was $\pm$ 10—12%, the stimulatory effect observed at 1 h post--treatment may not be significant.

The above in-vitro data transposed to the in-vivo situation would mean that Sermion has no effect on the synthesis of ribosomal-like RNA, whereas it may have a slight stimulatory effect on the synthesis of messenger-like RNA in the early phase of its action. However, due to a very high complexity of polymorphic structure and a high turn-over rate of macromolecules in the brain, this interpretation may, or may not, be valid. The increase of protein synthesis observed in short time intervals after Sermion treatment could be due to a number of other reasons, such as increased half-life of mRNA,

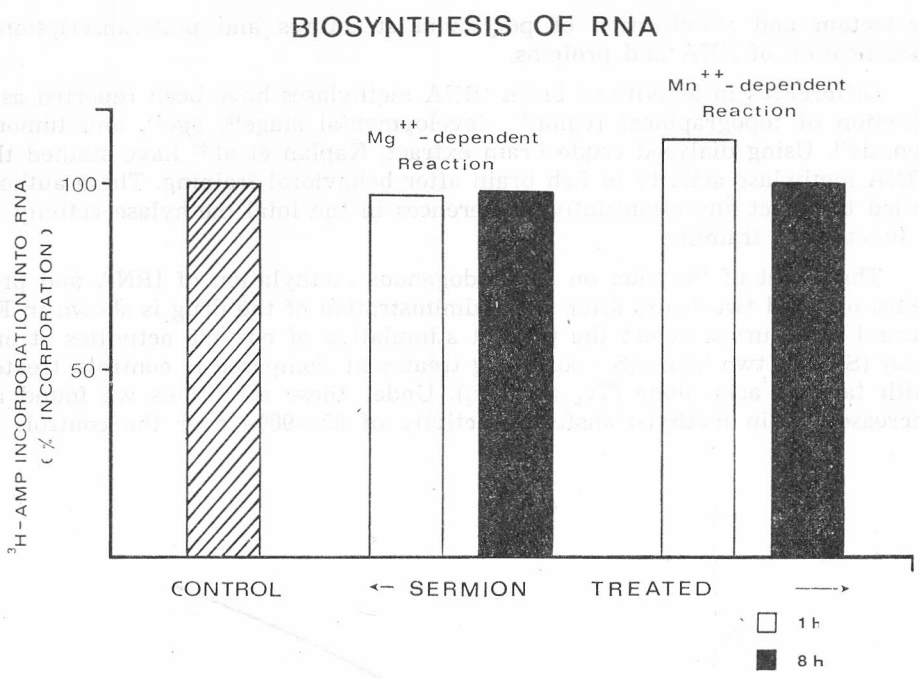

Figure 3. In-vivo effect of Sermion on the biosynthesis of RNA, catalyzed by Mg^{++}-ions, or Mn^{++}-ions supplemented with ammonium sulfate. Experimental details as described in the text and under Experimental: 100% incorporation designates 4085 c.p.m./mg of nuclear DNA. Eeach value is the arithmetic mean of five different experiments.

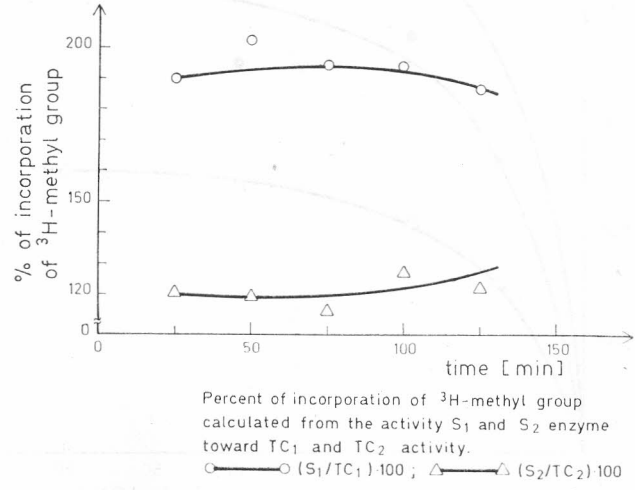

Figure 4. In-vivo effect of Sermion on the endogenenous methylation of proteins and tRNA from mouse brain: S_1 and S_2 are the enzyme preparations from Sermion-treated animals (S_1 = 1 h post-treatment period; S_2 = 2 h post-treatment period). TC_1 and TC_2 are the enzyme preparations from control animals treated with the solvent (TC_1 = 1 h post-treatment; TC_2 = 2 h post-treatment). Abscisa = incubation time i nmin.; ordinate = percent incorporation of ^{3}H-methyl groups over controls. All values were calculated as c.p.m./mg of protein present in the enzyme fraction.

protection and stabilization of polysomal structures and posttranscriptional modification of RNA and proteins.

Differences in activity of brain tRNA methylases have been reported as a function of topographical region[18], developmental stage[19], age[20], and tumorigenesis[21]. Using dialyzed crude brain extract, Kaplan et al.[22] have studied the tRNA methylase activity in fish brain after behavioral training. These authors failed to detect any quantitative differences in the total methylase activity as a function of training.

The effect of Sermion on the endogenous methylation of tRNA and proteins, one and two hours after i. p. -administration of the drug is shown in Figure 4. Tre curves depict the percent stimulation of enzyme activities at one hour (S_1) and two hours (S_2) post-drug treatment, compared to controls, treated with tartaric acid alone (Tc_1 and Tc_2). Under these conditions we found an increased brain methyl-transferase activity of 85—90% over the controls at

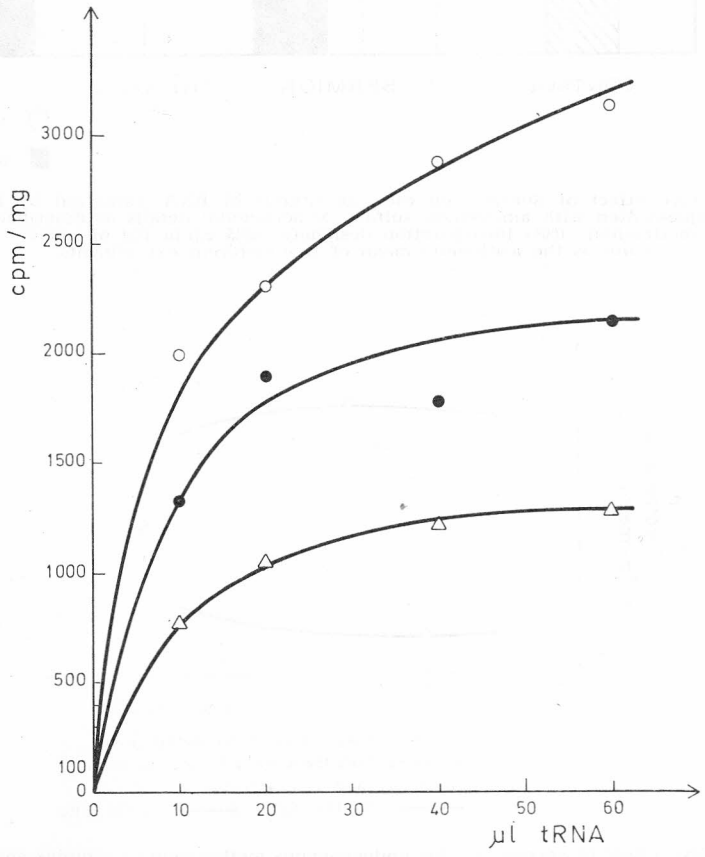

Figure 5. Methylation of exogenously added tRNA by brain preparations from mice of different age groups: methyltransferases were isolated from mice weighing 5g, 10g and 20g. The experimental details are described under Experimental.
○—○ = methyltransferase (5g); ●—● = methyltransferase (10g); △—△ = methyltransferase (20g)

1 hour post-treatment; however, the stimulatory effect was not significant after 2 hours of drug treatment. The stimulatory effect was evident at 25 and 125 minutes of incubation.

In another set of experiments the kinetics of methylation at various tRNA concentrations were studied in brain preparations from mice of different age groups. For these experiments, mice were divided by weight into three groups; methyl transferases were isolated from mice weighing 5 g, 10 g and 20 g. As follows from Figure 5, the ability of mouse brain preparations to catalyze the methyl group incorporation into tRNA decreases as the age of the animals increases. Using a dialyzed 160,000 x g supernatant, Johnson et al.[20] have recently studied the tRNA methylase activity in neonatal and mature mouse brains. When increasing amounts of the dialyzed supernatants from young and adult mouse brain were added to reaction mixtures, the saturation kinetics was found to be considerably different; at concentrations above 50 μg protein, the methylase activity of brain preparations from adult mice reached a plateau, whereas the methylase activity of neonatal brain preparations showed a linear rise. In all these experiments the endogenous values for each group of animals were subtracted; the endogenous value for 5 g mice was 930 c.p.m/mg protein; for 10 g mice 714 c.p.m/mg protein; and for 20 g mice 677 c.p.m/mg protein.

The in-vitro effects of Sermion on the endogenous mehylation by brain preparations from mice of different age groups (5 g, 10 g and 20 g body weigh) is shown in Figure 6. As follows from Figure 6, Sermion at 10^{-4} M stimulates the endogenous methylation of tRNA and proteins, in brain preparations from mice of all age groups. However it is remarkable that the stimulating effect of sermion in brain preparations from older animals (10g and 20 g body weight) is higher than that observed in the 5 g animals. Under similar experimental conditions we failed to observe any effect of sermion (10^{-4} M) on the methylation of exogenously added tRNA by brain preparations from mice of various age groups. Cummins et al.[23] have recently reported that a sizable

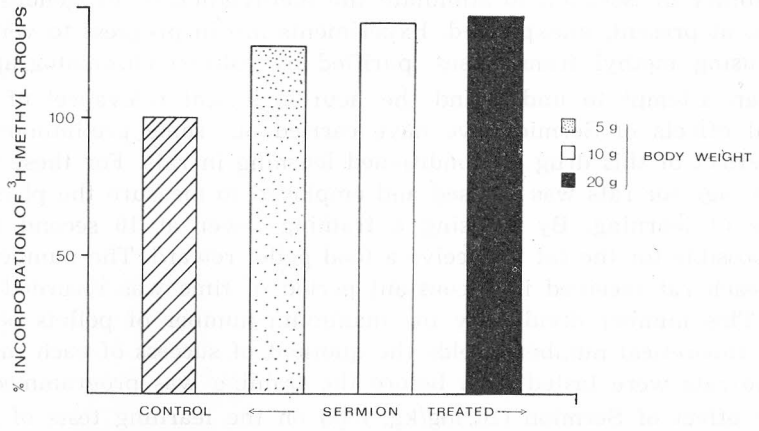

Figure 6. In-vitro effect of Sermion on the endogeneous methylation of brain preparations from mice of various age groups: drug concentration = 10^{-4} M; other details are described under Experimental.

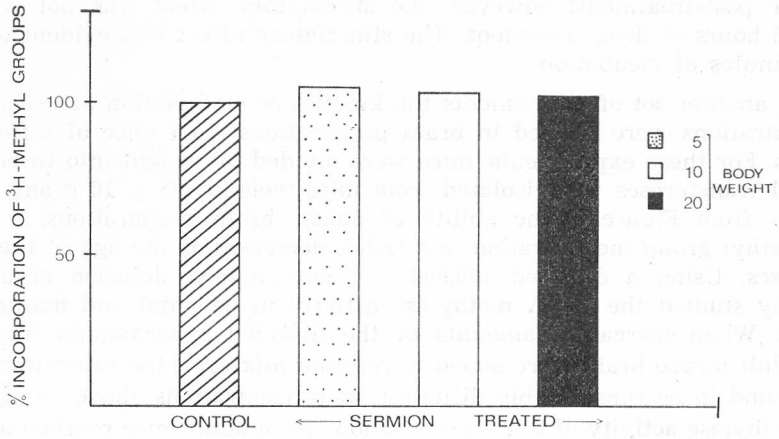

Figure 7. In-vitro effect of sermion (10^{-4} M) on the methylation of exogenously added tRNA by brain preparations from mice of various groups. For experimental details see Experimental.

portion of the endogenous methylation in rat brain cortex involves homologous tRNA. It remains to be investigated whether the endogenous methylation in various age groups is entirely due to homologous tRNA, or are proteins also involved. The pattern of methylation of RNA in the peripheral nerve of the chick during development has been studied by Mezei and Hu[24]. According to their observations the incorporation of methyl groups from methionine was the highest in the heavy RNA species of the 14-day embryonic nerve. In contrast, in the nerves of 3-day-old chicks, methylation was entirely localized to tRNA species. The situation in our experiments is different, since no heavy RNA fraction was present in the supernatants (150,000 x g) used by us.

The in vivo and in-vitro effects of Sermion on the methylation of endogenous tRNA and proteins are similar, in the sense that both are stimulated. The inability of Sermion to stimulate the methylation of exogenously added tRNA is, at present, unexplained. Experiments are in progress to verify these effects using methyl transferases, purified by column chromatography.

In an attempt to understand the neurobiological relevance[1] of the biochemical effects of Sermion, we have carried out some preliminary studies on the effect of this drug on conditioned learning in rats. For these studies a training cage for rats was devised and employed to measure the physiological response of learning. By pressing a training lewer at 10 second intervals, it was possible for the rat to receive a food pellet reward. The number of food pellets each rat received in a constant period of time was recorded automatically. This number divided by the maximum number of pellets possible to receive (theoretical number) yields the quotient of success of each individuals test. The rats were fasted 16 h before the training was programmed.

The effect of Sermion (10 mg/kg, i. p.) on the learning tests of rats (150 g body weight, all males, Sprague-Dawley) is shown in Table I. The values indicate the percent of success (quotient $\times$ 100) in the different groups.

TABLE I

Effect of Sermion on the Percent Success of Rats Subsjected to Conditioned Learning

Treatment/1 mg/100 g (kind of injected drug)	Time after injection/h	success[a]/%
NaCl	1	64.9
„	24	65
Tartaric acid	1	72.7
„	24	66.7
Sermion	1	53.7
„	2	92
„	24	83.4

[a] Success: number of pellets rat received in the training cage, divided by the number of pellets possible to receive (theoretical number).

As follows from Table I, the animals of the control group treated with NaCl and tartaric acid solutions (2 animals each) showed a quotient of 65—72%. The animals treated with Sermion exhibited a variable quotient, depending upon the post treatment period. One hour after the drug treatment the quotient was lower than in the controls, however, at 2 and 24 h post-treatment the quotients were significantly higher. We observed that the animals in the 1st hour after drug treatment were »very tired«, which could have been due to the hypotensive effect of the drug at this dose. This might explain the low quotient at 1 h.

REFERENCES

1. P. Jakoubek, *Brain function and macromolecular synthesis* (1974), Published by Pion Limited, London.
2. K. Kanig, in: *Memory and transfer of information,* Edt. H. P. Zippel, Plenum Press (1973), pp. 571.
3. P. Pazzagglia, *Riv. Sper. Freniat. Med. Leg. Alienazioni Ment.* **95** (1972) 348.
4. E. Baldoni, P. Serentha, S. Cuttin, and G. Galetti, *Gazz. Int. Med. Chir.* **75** (1971) 965.
5. L. R. DeChatelet and H. J. McDonald, *Proc. Soc. Exp. Biol. Med.* **127** (1968) 415.
6. O. H. Lowry, N. J. Rosebrough, A. L. Farr, and R. J. Randall, *J. Biol. Chem.* **193** (1951) 265.
7. J. R. Tata, *Progr. Nucl. Acid Res. Mol. Biol.* **5** (1966) 191.
8. C. C. Widnell and J. R. Tata, *Biochem. Biophys. Acta* **87** (1964) 531.
9. K. Burton, *Biochem. J.* **62** (1956) 315.
10. H. Gross, Personal Communication.
11. C. Vesco and A. Guiditta, *J. Neurochem.* **15** (1968) 81.
12. J. W. MacInnes, E. H. McConkey, and K. Schlesinger, *J. Neurochem.* **17** (1970) 457.
13. A. Dunn, *Brain Res.* **35** (1971) 254.
14. S. H. Appel, W. Davis, and S. Scott, *Science* **157** (1967) 836.
15. H. R. Dellweg, R. Gerner, and A. Wacker, *J. Neurochem.* **15** (1968) 1109.
16. W. H. Gispen, D. Dewied, P. Schottman, and H. S. Jansz, *J. Neurochem.* **17** (1970) 751.
17. F. Arcamone, Personal Communication.
18. K. R. Swiatek, D. G. Streeter, and L. N. Simon, *Biochemistry Easton* **10** (1971) 2563.
19. T. N. Simon, A. J. Glasky, and T. H. Rejal, *Biochim. Biophys. Acta* **142** (1967) 99.

20. T. C. Johnson, R. A. Mathews, and L. Chou, *J. Neurochem.* **23** (1974) 489.
21. G. Viale, *Cancer Res.* **31** (1971) 605.
22. B. B. Kaplan, W. M. Riedesel, and J. L. Sirlin, *J. Neurochem.* **22** (1974) 177.
23. C. J. Cummins, C. E. Salas, and O. Z. Sellinger, *Brain Res.* **96** (1975) 407.
24. C. Mezei and Y. W. Hu, *J. Neurochem.* **19** (1972) 2071.

SAŽETAK

Djelovanje 1,6-dimetil-8β-(bromizonikotinoil-oksimetil)-10α-metoksiergolina (Sermion^{R}) na neke molekularne procese u mozgu

B. Mildner, A. Paul i P. Chandra

Studirano je djelovanje Sermiona^{R} na celularnu sintezu proteina u mozgu miša in vivo, nakon intraperitonealne primjene doza od 50 μg. Uzorci mozga ispitani u različitim vremenskim razmacima nakon primjene Sermiona^{R} pokazali su kratkotrajno povećanje ugradnje ^{3}H-leucina u proteine, nakon čega se ugradnja normalizirala, dosegavši kontrolne vrijednosti nakon 18 sati. Pod sličnim uvjetima Sermion^{R} nije pokazao utjecaj na sintezu RNA kataliziranu ionima Mg^{2+} ,ali je opaženo blago stimuliranje sinteze RNA aktivirane s pomoću Mn^{2+}.

Miševi koji su primili intraperitonealne injekcije od 50 μg Sermiona^{R} pokazali su povećanu aktivnost metiltransferaze, a maksimalna je aktivnost bila dosegnuta 1 sat nakon primjene. U zasebnom nizu eksperimenata pokazalo se da sposobnost preparata mozga miša za ugradnju metilne skupine u tRNA dodanu izvana opada sa starošću životinje. Dodatak 10^{-4} M Sermiona inkubacijskom mediju pojačao je endogeno metiliranje, posebno u starijih životinja. Nasuprot tomu, Sermion^{R} nije utjecao na sposobnost metiliranja izvana dodane tRNA ,bez obzira na dob životinje.

U pokušaju da se razumije fiziološko značenje biokemijskog efekta Sermiona^{R}, proučavano je njegovo djelovanje na uvjetno učenje u štakora. Preliminarni podaci ukazuju da taj spoj povećava kvocijent učenja, no tek daljnji eksperimenti pokazat će ispravnost toga zaključka.

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Prispjelo 26. travnja 1976.