2. M. Kates, Techniques in Lipodology [Russian translation], Moscow (1975).
3. G. V. Kornilaeva, V. A. Slepushkin, and A. G. Budrinskaya, Vopr. Virusol., No. 1, 35 (1986).
4. Yu. M. Krasnopol'skii and V. I. Shvets, Cellular Bases of Antitumor Immunity [in Russian], Moscow (1985), p. 44.
5. M. I. Prokhorova (ed.), Methods in Biochemical Research [in Russian], Leningrad (1982), p. 101.
6. L. D. Bergel'son, É. V. Dyatlovitskaya, Yu. G. Molotkovskii, et al., Preparative Lipid Biochemistry [in Russian], Moscow (1981).
7. L. D. Bergel'son (L. D. Bergelson), A. G. Bukrinskaya, N. V. Prokazova, et al., Eur. J. Biochem., 128, Nos. 2-3, 467 (1982).

SPECIFIC BINDING OF (+)-^{3}H-SKF 10047 BY MOUSE SPLENOCYTES

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The compound SKF 10047 (N-allylmetazocine), when administrated to laboratory animals, induces several characteristic responses, namely: a psychotic state, ataxia, inhibition of respiration, dilatation of the pupils, and tachycardia. On the basis of experiments on spinal dogs, Martin and co-workers [5] classed this compound as an agonist of one type (σ) of opioid receptors. In the CNS, stereoisomers of N-allylnormetazocine bind with different receptors: the (−)-isomer interacts mainly with μ-receptors and depresses the effects of morphine, whereas the (+)-isomer binds with type σ receptors and has a psychotomimetic action [3, 10-12]. Specific binding of SKF 10047 has been found in several peripheral organs [1]: in the liver, kidneys, spleen, heart, and gonads. A detailed study of binding sites of SKF 10047 in the rat liver has demonstrated their similarity with the σ-receptors of the brain.

In this investigation specific binding of the tritium-labeled (+)-isomer of SKF 10047 with mouse splenocytes was demonstrated. A study of the binding sites of (+)-SKF 10047 with splenocytes showed that they have a definite similarity to the σ-receptors of the mammalian brain and to binding sites on membranes isolated from rat liver.

EXPERIMENTAL METHOD

Male CBA, C57BL/6, BALB/c and, predominantly, DBA/2 mice were used in the experiments. Animals of the first two lines are known to be resistant to stress, whereas mice of the last two lines are highly susceptible to stress-induced lesions.

Mouse splenocytes were obtained by the method described previously [2] and were resuspended in buffer: Tris-HCl 20 mM, sucrose 0.25 M (pH 7.4), at 25°C (buffer 1).

Binding of (+)-SKF 10047 with splenocytes was studied as follows. Samples of 500 μl in volume contained 10^7 cells and also different concentrations of labeled ligand and of substances hypothetically competing with it for specific binding sites on the splenocytes. All components of the mixture were dissolved or suspended in buffer 1. Incubation was carried out in plastic test tubes at 37 or 0°C. After the end of incubation 1 ml of buffer 1, cooled to 4°C, was added to the sample, the mixture was filtered in vacuo through GF/C filters (Whatman, England), and the filters were washed with 10 ml of cold buffer, dried in air, transferred into scintillation mixture (toluene 2 liters, Triton X-100 1 liter, PPO 22.5 g, POPOP

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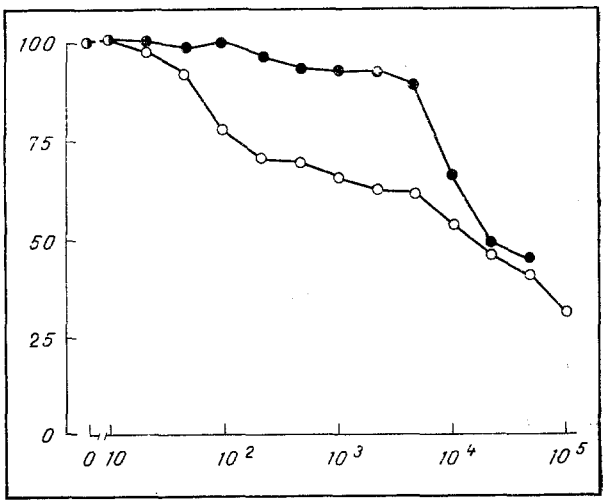

Fig. 1

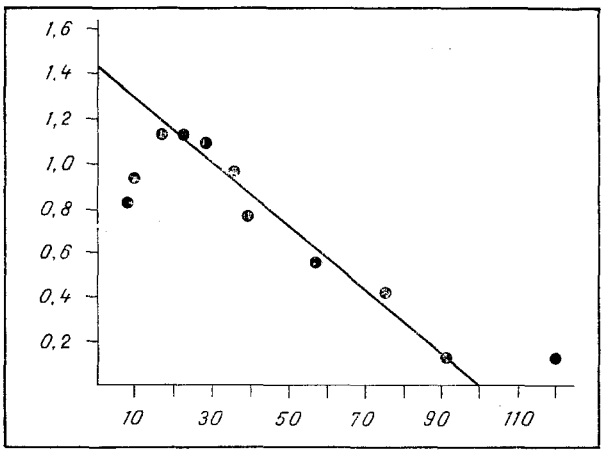

Fig. 2

Fig. 1. Inhibition of binding of (+)-^{3}H-SKF 10047 with mouse splenocytes by stereoisomers of bremazocine. Abscissa, concentration of stereoisomer (in nM); ordinate, inhibition of binding (in %). Empty circles – inhibition by (+)-bremazocine; filled circles – inhibition by (–)-bremazocine. Splenocytes of DBA/2 mice were incubated for 15 min at 37°C in the presence of 10 nM (+)-^{3}H-SKF 10047 and of various concentrations of bremazocine stereoisomers.

Fig. 2. Isotherm of binding of (+)-^{3}H-SKF 10047 by mouse splenocytes in Scatchard's coordinates. Abscissa, B (in fmoles/mg protein); ordinate, B/F (in fmoles/mg protein/nM). Splenocytes of DBA/2 mice were incubated for 15 min at 37°C in the presence of different concentrations of (+)-^{3}H-SKF 10047. Nonspecific binding was determined in the presence of 10 μM SKF 10047.

2.25 g) and after extraction for 24 h, the radioactivity of the samples was determined by means of a liquid scintillation spectrometer. Binding with the filters in the absence of cells was determined for each concentration of labeled ligand. The results obtained were subtracted from the value of binding in the presence of splenocytes.

EXPERIMENTAL RESULTS

Preliminary experiments showed that at both 0°C and 37°C (+)-^{3}H-SKF 10047 bound with splenocytes of DBA/2 mice over a wide range of concentrations of the labeled ligand. Addition of the unlabeled racemate of SKF 10047 in a concentration of 10 μM inhibited much of the binding. Binding not depressed in the presence of 10 μM unlabeled ligand was considered to be nonspecific, whereas the difference in binding in the absence and presence of labeled SKF 10047 was considered to be specific. The experiments showed that with labeled ligand in a concentration of 10 nM, specific binding left 45-55% of the total and it changed as a linear function of splenocyte concentration over the range from 1 to $15 \cdot 10^6$ cells per sample. These data were used to select the conditions for the subsequent experiments.

The kinetics of association and dissociation of (+)-^{3}H-SKF 10047 was studied at 0 and 37°C. Association of the ligand at both temperatures took place very quickly. The maximal level of total and specific binding was reached in 20 min at 0°C and in 10 min at 37°C. Dissociation also took place rapidly: binding was reduced tenfold after 10-15 min at 0°C and a hundredfold 3-4 min after dilution of the incubation mixture with buffer 1 at 37°C. The ligand dissociated virtually completely. Thus kinetic equilibrium between binding and dissociation was established in 10 min at 37°C and in 20 min at 0°C. In the experiments whose results are shown in Figs. 1 and 2, and in Table 1, an incubation time of 15-20 min was used, i.e., the experiments were conducted under conditions of kinetic equilibrium.

To characterize the specificity of SKF 10047 binding sites on the splenocytes experiments were carried out to study inhibition of binding of (+)-^{3}H-SKF 10047 by several biologically active compounds. These results are shown in Fig. 1 and Table 1. Binding of (+)-^{3}H-SKF 10047 with mouse spleen cells was inhibited by increasing concentrations of unlabeled SKF 10047, at least up to 10^{-4} M. The curves of displacement of (+)-^{3}H-SKF 10047 by the unlabeled racemate of SKF 10047, by (+)-bremazocine (Fig. 1), and by dextrorphan were biphasic: part of the binding was inhibited by low concentrations of these substances (10^{-7}-$5 \cdot 10^{-7}$ M),

TABLE 1. Inhibition of Binding of (+)-^{3}H-SKF 10047 with Mouse Splenocytes by Various Substances

Compound	IC_{50}, M	
	4 °C	37 °C
(±)-SKF 10047	$8{,}2 \cdot 10^{-5}$	$1{,}1 \cdot 10^{-6}$
(+)-Bremazocine	$2{,}5 \cdot 10^{-5}$	$2{,}2 \cdot 10^{-6}$
(−)-Bremazocine	$1{,}2 \cdot 10^{-5}$	$1{,}3 \cdot 10^{-5}$
Levallorphan	—	Over $5 \cdot 10^{-5}$
Dextrorphan	—	$9{,}4 \cdot 10^{-7}$
Morphine	—	$1{,}1 \cdot 10^{-4}$
Naloxone	—	$9{,}3 \cdot 10^{-5}$
Phencyclidine	—	$5{,}1 \cdot 10^{-6}$
Propranolol	—	$1{,}5 \cdot 10^{-7}$
Haloperidol	—	$1{,}4 \cdot 10^{-8}$

Legend. Splenocytes of DBA/2 mice were incubated in the presence of 10 nM (+)-^{3}H-SKF 10047 and different concentrations of the test substances. The data shown are mean values of the results of two independent experiments. The results of the independent experiments differed by not more than 20%. Haloperidol inhibited binding of the label only to the level recorded in the presence of 10^{-5} M unlabeled (±)-SKF 10047. For that reason IC_{50} in this case was calculated for binding inhibited by 10^{-5} M unlabeled (±)-SKF 10047.

and this was followed by a region in which an increase in the concentration of the unlabeled ligand had virtually no effect on binding, and further inhibition took place only in the presence of very high concentrations (above 10 μM). (−)-Bremazocine (Fig. 1) and levallorphan (the latter, unlike dextrorphan, interacts with high affinity with opioid μ-receptors) displaced (+)-^{3}H-SKF 10047 much less strongly. Binding of the label was inhibited by low concentrations of propranolol and partially, by very low (about 10^{-8} M) concentrations of haloperidol. It will be clear from Table 1 that it was also depressed by phencyclidine. Morphine and naloxone had hardly any effect on it. If the temperature was lowered the ability of the SKF 10047 racemate and of (+)-bremazocine to depress binding of (+)-^{3}H-SKF 10047 fell considerably, but the inhibitory activity of (−)-bremazocine was virtually unchanged. When the results given in Table 1 were calculated, specific binding was taken to be the difference between binding in the absence and in the presence of 100 μM SKF 10047. Unlike a concentration of 10 μM, this last concentration depressed both high- and low-affinity binding of the label with the splenocytes.

We know that the (+)-isomer of SKF 10047 binds specifically with σ-opiate receptors in the mammalian brain [9-12]. This binding is selectively inhibited by (+)-bremazocine, haloperidol, propranolol, phencyclidine, and dextrorphan. All these compounds are known to interact with the σ-receptors of the CNS. The classical opiates, namely morphine, naloxone, and levallorphan, do not interact with σ-receptors. Considering the facts described above, it can be tentatively suggested that binding of (+)-^{3}H-SKF 10047 with splenocytes, inhibited by low concentrations of SKF 10047 and (+)-bremazocine, is binding with sites similar to σ-receptors. Binding inhibited by high concentrations of these substances probably reflects interaction of the ligand with certain other sites or its nonspecific association with the cells. It also follows from Table 1 that high-affinity sites similar to σ-receptors are found only at a physiological temperature. In fact, IC_{50} (the concentration in which specific binding is inhibited by 50%) for SKF 10047 and for (+)-bremazocine increased tenfold when the temperature was lowered. IC_{50} for (−)-bremazocine was unchanged, evidence that this ligand inhibits binding that is only weakly dependent on temperature, and, perhaps, nonspecific sorption.

The isotherm of specific binding of (+)-^{3}H-SKF 10047 with DBA/2 mouse splenocytes is shown in Fig. 2 as a Scatchard plot. Nonspecific binding in this case was determined in the presence of 10 μM SKF 10047 (this concentration, as follows from Fig. 1, inhibits stereoselective

binding of the label, i.e., conjecturally it inhibits association with sites of the σ-receptor type). The isotherm is complex in shape. At low concentrations of the label the curve is convex (apex uppermost), indicating the possibility of positive cooperative interaction between ligand-binding sites. With an increase in concentration of the labeled ligand suturation of the receptors is observed and the isotherm on a Scatchard plot becomes virtually a straight line. The dissociation constant (K_d) of the ligand-receptor complex, determined graphically on the linear region, is 45 nM, and the concentration of binding sites is 70 fmoles/10^6 cells, or about 100 molecules per cell. Such a low concentration of binding sites indicates that σ-receptors are present only on a certain splenocyte subpopulation. In other analogous experiments similar values were obtained for the binding parameters (K_d from 20 to 80 nM and the concentration of sites from 30 to 100 fmoles per 10^6 cells). Isotherms of binding of (+)-^{3}H-SKF 10047 with splenocytes of CBA, C57BL/6, and BALB/c mice were similar to those obtained for cells of DBA/2 mice.

The results obtained in this investigation are evidence that mouse splenocytes (possibly a certain subpopulation of these cells) carry binding sites for (+)-SKF 10047 in several properties to σ-receptors. As has already been stated, these receptors were found previously in the CNS. Zukin's group obtained evidence of the heterogeneity of specific binding sites of (+)-SKF 10047 in the brain [9]. According to data obtained by these workers, a small number of high-affinity (K_{d_1} = 3.6 nM) and low-affinity (K_{d_2} = 153 nM) receptors of this benzomorphan exist in the rat and mouse brain. The (+)-isomers of benzomorphans and phencyclidine interact with low-affinity sites, (+)-isomers of benzomorphans and the neuroleptic haloperidol interact with high-affinity sites. In the present experiments, stereoselective temperature-sensitive binding of (+)-^{3}H-SKF 10047 was inhibited by both these substances, although by phencyclidine in quite high concentrations. Because of the complex character of the isotherm of specific binding of the label, it is impossible to draw any unequivocal conclusion regarding the number of types of binding sites on splenocytes. Values of K_d obtained for the linear region of the isotherm occupy an intermediate position between values of K_{d_1} and K_{d_2} given in the publication cited above. It must be pointed out that values of K_d for the complex of central σ-receptors with (+)-^{3}H-SKF 10047, according to data in the literature, vary considerably [9-12]. In order to obtain a final answer to the question of the degree of homogeneity of binding sites of (+)-^{3}H-SKF 10047 on splenocytes, additional investigations are needed.

It was recently shown that SKF 10047 binds with homogenates of various internal organs [1]. A careful study of binding sites in the liver demonstrated their similarity to σ-receptors. These observations and our own results point to the possible presence of σ-receptors not only in the CNS, but also at the periphery.

The functions of the σ-receptors are not clear. We know, however, that they mediate several psychotomimetic responses in animals and man. SKF 10047 can change the blood levels of corticosterone and prolactin [6, 7]. Data have been obtained in recent years to show that the same biological active substances, including classical neurotransmitters (catecholamines, for example) and regulatory peptides, through their interaction with specific receptors on neurons and on endocrine and immune cells, can integrate the activity of the three most important regulatory systems — nervous, endocrine, and immune [8]. Our own results are evidence that endogenous ligands of σ-receptors, which have not yet been isolated, may also belong to the list of these substances.

Our own results agree to some degree with data published by Marks and Medzihradsky [4], who found binding of another benzomorphan, namely pentazocine, with rat leukocytes. Binding of pentazocine, which the authors cited interpreted as facilitated uptake, obeyed a Michaelis–Menten kinetics with K_m = 40 μM, was characterized by a very rapid kinetics (the time taken to reach equilibrium was 5 min), it was completely reversible, it was unchanged in the presence of sodium, it was nonstereoselective, and depended on temperature. It is easy to see that the properties of the benzomorphan carrier described in the publication cited are similar in many respects to the properties of the low-affinity binding site for (+)-^{3}H-SKF 10047 on splenocytes.

LITERATURE CITED

1. N. N. Samovilova, K. N. Yarygin, and V. A. Vinogradov, Bioorg. Khim., 11, No. 10, 1380 (1985).
2. G. T. Sukhikh, L. V. Van'ko, F. Z. Meerson, and B. B. Fuks, Vest. Akad. Med. Nauk SSSR, No. 11, 16 (1983).

3. B. L. Largent, A. L. Gundlach, and S. H. Snyder, Proc. Natl. Acad. Sci. USA, 81, No. 15, 4983 (1984).
4. M. J. Marks and F. Medzihradsky, Mol. Pharmacol., 10, No. 5, 837 (1974).
5. W. R. Martin, C. G. Eades, J. A. Thompson, et al., J. Pharmacol. Exp. Ther., 197, No. 3, 517 (1976).
6. R. Pechnick, R. George, and R. E. Poland, J. Pharmacol. Exp. Ther., 232, No. 1, 163 (1985).
7. R. Pechnick, R. George, and R. E. Poland, J. Pharmacol. Exp. Ther., 232, No. 1, 170 (1985).
8. C. B. Pert, M. R. Ruff, R. J. Weber, and M. Herkenham, J. Immunol., 135, No. 2, 820 (1985).
9. R. Sircar, R. Nichtenhauser, J. R. Ieni, and S. R. Zukin, J. Pharmacol. Exp. Ther., 237, No. 2, 681 (1986).
10. T.-P. Su, J. Pharmacol. Exp. Ther., 223, No. 2, 284 (1982).
11. S. W. Tam, Proc. Natl. Acad. Sci., USA, 81, No. 17, 5618 (1984).
12. S. W. Tam, Eur. J. Pharmacol., 109, No. 1, 33 (1985).

ACTION OF MYELOPEPTIDES ON DNA AND TOTAL PROTEIN SYNTHESIS IN CELLS OF MOUSE LYMPHOID ORGANS

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Great interest has recently been aroused by the special class of low-molecular-weight (mol. wt. about 2 kilodaltons) peptides of bone marrow origin, namely myelopeptides (MP), discovered by Petrov and co-workers [2-4, 8]. The antibody-stimulating and opioid properties of MP are well known [3, 4, 8], although there are virtually no data on the possible action of MP on integral parameters of cell metabolism such as synthesis of chromosomal DNA and of total protein. In view of the realistic prospects for the use of MP as a therapeutic preparation (B-activin, myelopid), the need for obtaining such data will be evident. At the same time, there is reason to suppose that MP may also have diverse functions [2, 4, 8].

The aim of this investigation was to study the action of MP on replicative DNA synthesis and protein synthesis (without histones) in cells of mouse lymphoid organs.

EXPERIMENTAL METHOD

Cells from nonimmune (except lymph nodes) lymphoid organs of (CBA × C57BL/6)F_1 mice were used. MP were isolated from pig bone marrow by the usual method [1]. The cells were incubated in a concentration of $3 \cdot 10^6$/ml for 2 days in medium RPMI-1640, with essential additives [5] in the presence of MP. The dose of MP in the incubation medium (50 μg/ml) was chosen experimentally within the range from 1 to 100 μg/ml. In some cases 2-mercaptoethanol (ME) was added to the incubation medium in a concentration of $5 \cdot 10^{-5}$ M with or without MP. ^{3}H-thymidine (2 μCi/ml, 19 Ci/mole) together with ^{14}C-amino acids (1 μCi/ml, chlorella digest, Czechoslovakia) were added to the culture 4 h after the end of incubation in 24-well channels (Nuclon, Denmark) in an atmosphere of 5% CO_2 at 37°C. After the end of incubation the cells were transferred into centrifuge tubes and washed twice in an excess of medium 199 in the cold to remove nonspecifically adsorbed radioactive label. The washed cells were suspended in 2 ml of cold water, and the carrier protein (70 μg/ml) and TCA to a final concentration of 10% were added to the suspensions. After the residues had formed the samples were shaken vigorously in the cold for 30 min. Under these conditions histones go into

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