EFFECT OF MORPHINE ON ADENYLATE CYCLASE ACTIVITY IN LYMPHOCYTES OF HEALTHY CONTROLS, ALCOHOLICS, AND OPIATE ADDICTS

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The use of radioactive receptor methods has recently proved the presence of binding sites for ligands of opioid receptors on immunocompetent cells of a heterogenous population [3, 14]. Data on the existence of such sites on human peripheral blood T lymphocytes were reported in [11, 12], the authors of which discovered specific binding sites for [^{3}H]-naloxone, similar in their properties to mu- and delta-types of opioid receptors. One of the mechanisms of action of opioids on metabolism of cells including immunocompetent cells, is the regulation of activity of the plasma membrane cyclase enzymes [2, 4, 9]. For this reason, changes in the character of activity of these systems by opioids may be indirect evidence of changes in the state of the corresponding binding sites.

In research published previously the action of opioids on cAMP levels was assessed by the study of a small number of healthy human lymphocytes. In the accessible literature there is no information on the study of opiate-dependent adenylate cyclase (AC) in diseases in whose pathogenesis disturbances of the opiate system play a role and, in particular, in alcoholism and opium addiction [1, 5]. If such research were undertaken it would make an important contribution to our knowledge of the pathogenesis of these forms of dependence in man, more especially because information is increasingly being published to show that cyclase enzymes play a role in the mechanisms of development of tolerance to alcohol and opiates, depending on them [8, 10, 13]. However, most of the conclusions are based on the results of animal experiments.

The aim of the present investigation was to study the effect of morphine on AC activity in lymphocytes of healthy individuals, alcoholics, and opium addiction, using the opioid receptor blocker naloxone.

EXPERIMENTAL METHOD

Lymphocytes were isolated from 5-10 ml of heparinized blood on Ficoll–Verografin gradient by the method in [7]. The cell suspensions usually contained not fewer than $5 \cdot 10^6$ lymphocytes in 1 ml. Basal AC activity was measured as the quantity of cAMP formed during the incubation period, and was expressed in pmoles cyclic nucleotide/min of incubation/10^6 lymphocytes. The incubation mixture contained 50 mmoles Tris-HCl buffer (pH 7.4), 10 mmoles KCl, 20 mmoles $MgCl_2$, 6 mmoles theophylline, and 1 mmole ATP in a final volume of 0.5 ml [6]. The reaction was started by the addition of 0.1 ml of a suspension of lymphocyte membranes (equivalent to 0.5-1.0 million cells), and was stopped after incubation for 30 min at 37°C by the addition of 1 ml of 96° ethanol, followed by centrifugation at 16,000*g* for 10 min. The supernatant, containing cAMP, was dried at 70-80°C and stored at −20°C until required for determination. The cAMP content was determined by the use of commercial kits (Cyclic AMP Assay Kit, from Amersham, England). The effect of morphine (pharmaceutical preparation), naloxone (Sigma), and their combination on AC activity was determined by adding the drugs to the incubation mixture. The effect of the drugs was assessed as an action index (AI), calculated as the change of AC activity as percentage of the basal level. In preliminary experiments the dose of morphine of 10^{-7} M and an exposure of 30

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TABLE 1. Basal AC Activity of Lymphocytes and Effect of Morphine on it ($\bar{X} \pm S_{\bar{x}}$)

Group tested	Basal AC activity, pmoles/min/ 10^6 lymphocytes	Effect of morphine, AI_m, %
Healthy (n = 20)	0,40±0,11	—1,2±6,8
Alcoholics in withdrawal syndrome		
On admission	0,56±0,10	49,8±17,4
(n=16)	(t=1,07)	(t=2,95, p<0,01)
One week after	0,75±0,19	123,1±37,0
(n=16)	(t=1,67)	(t=3,67, p<0,001)
3-5 weeks after	0,92±0,30	50,1±24,1
(n=13)	(t=1,93)	(t=2,44, p<0,01)
Sober alcoholics	0,36±0,13	—42,8±14,7
(n=10)	(t=0,22)	(t=3,13, p<0,01)
Patients with opium addiction in withdrawal syndrome		
On admission	0,23±0,08	33,7±13,8
(n=9)	(t=1,01)	(t=2,55, p<0,02)
One week after	0,17±0,05	34,4±13,5
	(t=1,39)	(t=2,63, p<0,02)

Legend. Comparison with healthy group.

min were chosen, to enable the patients to be distinguished most reliably from healthy controls. The results were expressed as mean values with standard errors of the means. The significance of difference between the means was estimated by Student's t-test. Lymphocytes were obtained from 20 healthy blood donors (men, average age 33.4 ± 1.5 years), from 26 alcoholics (men, average age 37.6 ± 2.0 years), and 10 opium addicts (men, average age 27.2 ± 2.7 years), hospitalized in the All-Union Drug Addiction Research Center, to undergo a course of treatment. The average duration of the disease in the alcoholics was 12.8 ± 1.5 years, and in the opium addicts 4.7 ± 1.3 years. The patients were investigated at intervals: on admission (as a rule before treatment began), one week later, and again 3-5 weeks later (alcoholics). In the alcoholics the severity of the withdrawal syndrome was estimated in points, from 0 (absence of withdrawal symptoms) to 3 (severe withdrawal syndrome). Patients in the withdrawal syndrome were given detoxication treatment.

EXPERIMENTAL RESULTS

Basal AC activity and the effect of morphine (AI_m) are given in Table 1. The results show that by contrast with the controls, in the chronic alcoholics and opium addicts with a withdrawal syndrome the chosen dose of morphine (10^{-7} M) had a stimulating effect on AC activity of the lymphocytes. It must also be noted that in alcoholics admitted with the withdrawal syndrome, toward the end of the first week of their stay in hospital, the activating effect of morphine became even stronger, which may perhaps be partly due to the treatment given. In patients with opium addiction, dynamic changes in opiate-dependent AC were of a slight degree, despite the detoxication treatment, probably indicating reduction of reserve capacity of the AC system. The absolute value of the range of change in the effect of morphine (ΔAI_m) on AC activity in the course of one week in the alcoholics was 162.9 ± 33.0%, whereas in the opium addicts it was 30.4 ± 4.6% ($p < 0.01$).

In alcoholics admitted in a sober state morphine had an inhibitory effect on enzyme activity, possibly a characteristic feature of chronic alcoholic intoxication.

The data in Table 2 on the effect of morphine, naloxone, and a combination of both on AC of the lymphocytes demonstrate the abolition of the stimulating effect of morphine by naloxone. These results, together with data in the literature [2, 4] on the blocking of the action of opioids (Met-enkephalin, morphine) by naloxone on the cyclic nucleotide level in human peripheral blood lymphocytes in vitro are indirect evidence of the existence of corresponding receptors on lymphocyte membranes. Changes in the character of the modulating effect of morphine on AC activity in lymphocytes of alcoholics and opium addicts may be the result of the action of alcoholic and opium poisoning on that system.

TABLE 2. Effect (AI) of Morphine, Naloxone, and a Combination of Both on AC Activity of Lymphocytes ($X \pm S_x$)

Lymphocytes	AI, per cent		
	morphine (10^{-7} M)	naloxone (10^{-7} M)	morphine (10^{-7} M) + naloxone (10^{-7} M)
From healthy individuals (n = 8)	46,8±31,3	15,5±22.0 t=0,82	15,1±19,7 t=0,86
From alcoholics (n = 35)	74,5±24,3	13,2±18.3 (t=2,01, p<0,05)	6,6±10,5 (t=2,56, p<0,02)

Legend. Comparison of AI of morphine.

Calculation of coefficients of correlation between the biological parameters and the characteristics of the history of addiction in the alcoholics (n = 24) revealed significant positive correlations between the severity of the withdrawal syndrome on admission, on the one hand, and the effect of morphine on AC activity after one week (AI_m2; $r = 0.578$, $p < 0.01$) or with the absolute range of the change in this effect (ΔAI_m; $r = 0.696$, $p < 0.01$), on the other hand. Positive correlation also was found between the above-mentioned biological parameters and the duration of the last drinking bout ($r = 0.437$, $p < 0.05$ and $r = 0.450$, $p < 0.05$ respectively). In opium addicts (n = 10), negative correlation was found between the effect of morphine on AC activity on admission (AI 1) and the total duration of addiction ($r = -0.708$, $p < 0.05$), and also between ΔAI_m and tolerance to the drug ($r = -0.678$). Positive correlation also was found between the dose of the drug taken and AI_m2 ($r = 0.763$, $p < 0.05$).

The clinical-biological correlation thus revealed are further confirmation of the conclusion that assessment of the modulating effect of morphine on AC activity of human peripheral blood lymphocytes can give some idea of the possible changes in the state of the opiate-dependent cyclase systems also in cells of the CNS, involved in the pathogenesis of alcohol and opium dependence.

On the basis of these results the test of determining sensitivity of lymphocytic AC to morphine can be recommended as a means of estimating the severity of drug addiction of patients and for monitoring the efficacy of treatment.

LITERATURE CITED

1. I. P. Anokhina, A. M. Balashov, B. M. Kogan, and L. F. Panchenko, Vorp. Narkol., No. 3, 3 (1989).
2. A. A. Zozulya, É. Patsakova, and N. V. Kost, Vestn. Akad. Med. Nauk SSSR, No. 1, 28 (1982).
3. A. A. Zozulya and S. F. Pschenichkin, Progress in Science and Technology, Series: Immunology [in Russian], Vol. 25, All-Union Institute of Scientific and Technical Information, Moscow (1990), pp. 48-120.
4. N. V. Kost and A. A. Zozulya, Farmakol. Toksikol., No. 2, 44 (1983).
5. L. F. Panchenko and A. M. Balashov, Vestn. Akad. Med. Nauk SSSR, No. 8, 18 (1986).
6. I. P. Anokhina (I. P. Anokina), B. M. Kogan, B. Nickel, et al., Biomed. Biochim. Acta, **48**, 593 (1989).
7. A. Boyum, Scand. J. Immunol., Suppl., 9 (1976).
8. R. S. Duman, J. F. Tallman, and E. J. Nestler, J. Pharmacol. Exp. Ther., **246**, 1033 (1988).
9. T. Fulop, D. Kekessy, and G. Foris, Int. J. Immunopharmacol., **9**, 651 (1978).
10. W. A. Klee, G. Milligan, W. F. Simonds, and B. Tocque, Nat. Inst. Drug Abuse Res. Monogr. Ser., **54**, 109 (1984).
11. J. J. Madden, R. M. Donahoe, J. Zwemer-Collins, et al., Biochem. Pharmacol., **36**, 4103 (1987).
12. J. N. Mehrishi and I. H. Mills, Clin. Immunol. Immunopathol., **27**, 240 (1983).
13. L. B. Nagy, I. Diamond, and A. Gordon, Proc. Nat. Acad. Sci. USA, **85**, 6973 (1988).
14. H. Ovadia, P. Nitsan, and O. Abramsky, J. Neuroimmunol., **21**, 93 (1989).