reduction we found in the number of benzodiazepine receptors determines the animals' response to stress, which arises in rats with formed physical dependence on alcohol when deprived of ethanol. In particular, we know that stress, induced by various physical factors, is accompanied by a considerable decrease in the number of benzodiazepine receptors [7]. Without discussing the question of specificity of the patterns discovered in relation to the realization of mechanisms of formation of ethanol dependence, it can be tentatively suggested that weakening of functional activity of the GABA-benzodiazepine complex in animals predisposed to the development of experimental alcoholism is one of the neurochemical mechanisms of development of the abstinence syndrome.

LITERATURE CITED

1. Yu. V. Burov, T. I. Absava, A. B. Kampov-Polevoi, et al., Farmakol. Toksikol., No. 1, 50 (1981).
2. Yu. V. Burov, R. Yu. Ukhananov, and A. I.Maiskii, Byull. Éksp. Biol. Med., No. 9, 67 (1982).
3. I. V. Viglinskaya, in: Experimental and Clinical Psychopharmacology [in Russian], Moscow (1980), p. 107.
4. L. M. Aaronson, D. D. Himma, and M. Okamoto, J. Pharmacol. Exp. Ther., 221, 319 (1982).
5. G. Freund, Life Sci., 27, 987 (1980).
6. M. B. Lies and J. Bachman, Anal. Biochem., 47, 184 (1972).
7. J. Millan and T. Duka, Mod. Probl. Pharmacopsychiat., 17, 123 (1982).
8. L. Nestoros, Science, 209, 708 (1980).
9. S. M. Paul, P. J. Marangos, and P. Skolnick, Biol. Psychiat., 16, 213 (1981).

POTENTIATING ACTION OF SALTS OF BIVALENT AND TRIVALENT METALS ON THE ANALGESIC EFFECT OF MORPHINE

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KEY WORDS: morphine; salts of metals; potentiation; opiate receptors.

Evidence that La^{+++} cations potentiate morphine-induced analgesia has been published in [3, 5, 6]. The present writers showed previously [2] in experimens *in vitro* that La^{+++} cations, and also Mn^{++}, and Ni^{++} cations and lanthanides, interacting with specific binding sites of morphine and D-Ala^2-D-Leu^5-enkephalin (DADL) on rat brain membrane preparations, increase the affinity of opiate ligands for the corresponding receptors.

The aim of this investigation was to study the effect of salts of various metals ($MnCl_2$, $NiCl_2$, $GdCl_3$, and $LaCl_3$) on the analgesic effect of morphine. These salts were chosen because in experiments *in vitro* they were found to have the strongest activating action on affinity of opioid ligands for opiate receptors of rat brain membranes.

EXPERIMENTAL METHOD

The analgesic activity of the compounds in experiments on mice weighing 21-27 g was investigated after intracisternal [7] injection by the hot plate method (52°C). After preliminary assessment of the animals' response to painful contact stimulation 3 times, with an interval of 5 min, solutions of salts of the metals in isotonic NaCl solution were injected intracisternally in a dose of 10 µl. Morphine was again injected intracisternally in a dose of 2 mg per mouse 45 min after injection of the salts of the metals. In a parallel investigation

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TABLE 1. Effect of $MnCl_2$, $NiCl_2$, and $GdCl_3$ on Analgesic Effect of Morphine

Compound	Dose of compound, μg per mouse	Dose of morphine, μg per mouse	A/A_0	Sign test P	Wilcoxon's test P
$MnCl_2$	10	2	1,45	<0,2	<0,1
	20	2	2,0	<0,05	<0,05
	30	2	2,75	<0,01	<0,1
	40	2	2,85	<0,01	<0,05
$NiCl_2$	5	2	2,50	<0,05	<0,05
	20	2	3,0	<0,01	<0,1
$GdCl_3$	5	0,5	2,46	<0,05	<0,05
	5	0,75	2,30	<0,025	<0,1
	5	1,0	2,47	<0,025	<0,05
	5	2,0	2,40	<0,025	<0,05

Legend. P denotes significance of differences from control.

TABLE 2. ED_{50} for Salts of Metals with Respect to Ability to Potentiate Analgesic Effect of Morphine

Compound	ED_{50}, μg per mouse	K_{Me1}	K_{Me2}	K_{Me3}
		μM		
$NiCl_2$	2,2±0,7	6,6±0,5	5000	2,1±0,4
$GdCl_3$	3,8±1,2	11,0±2	1,4±0,3	5,0±1
$MnCl_2$	20,0±5,5	75±6	210±70	70±18
$LaCl_3$	—	275±35	40±12	210±48

morphine was injected after preliminary intracisternal injection of 10 μl of isotonic NaCl solution. The percentage of analgesia was calculated by the formula $A = (T_1 - T_0/T_2 - T_0) \cdot 100\%$, where T_0 and T_1 stand for the latent period of the response in the control and after injection of the preparation respectively, and T_2 denotes the longest possible time achieved (60 sec). The potentiating effect was determined as the ratio, in per cent, between the number of cases of analgesia (A/A_0) in the experimental (A) and control (A_0) groups, each consisting of eight animals, 60 min after injection of morphine. Statistical analysis of the results was carried out by Wilcoxon's nonparametric test [1] and by the nonparametric sign test [4]. The process of equilibrium binding of labeled ligands with opiate receptors in a preparation of rat brain membranes was investigated and parameters of the effect of cations of the metals on this process were determined by the methods described previously [2].

EXPERIMENTAL RESULTS

The experiments showed that $MnCl_2$, $NiCl_2$, $GdCl_3$, and $LaCl_3$, in doses of up to 30, 20, 5, and 10 μg, respectively, did not affect the threshold of the pain response, assessed by the hot plate method, and likewise did not cause any appreciable neurological disorders. Meanwhile these compounds, in the same doses, potentiated the analgesic action of morphine, when injected intracisternally in a dose close to the effective dose (ED_{50}) for this test, namely 2 μg per mouse (Table 1). It was also shown that $GdCl_3$ has an equal potentiating action (A/A_0) on the effect of morphine injected intracisternally within a dose range from 0.5 to 2 μg per mouse. Analysis of dependence of the potentiating effect on dose of the salt of the metal showed that it was hyperbolic in character. For instance, the maximal value of A/A_0 60 min after injection of morphine, when the above-mentioned salts of metals were used, irrespective of an increase in their dose, did not exceed 3.0. This rule means that the value of ED_{50} for the salt of the metal, corresponding to the dose giving half the maximal potentiating effect, can be calculated (Table 2). Of the salts of the metals studied, the most active was $NiCl_2$, for which ED_{50} was 2.2 ± 0.7 μg per mouse. The potentiating action of $MnCl_2$ was manifested as an increase in the number of cases of analgesia immediately after injection of morphine (at the 15th minute), and also as prolongation of its antinociceptive action (Fig. 1).

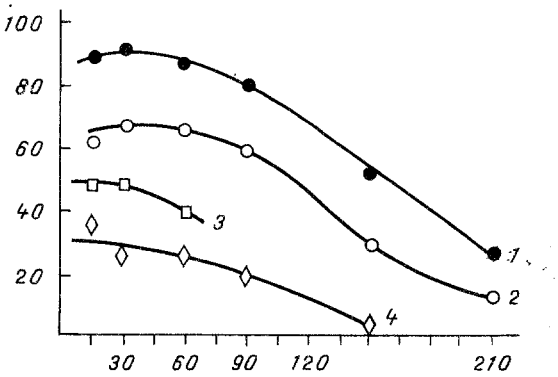

Fig. 1. Effect of $MnCl_2$ on antinociceptive effect of morphine. 1, 2, 3) $MnCl_2$ in doses of 30, 20, and 10 μg per mouse, respectively. 4) Control. Abscissa, time (in min); ordinate, analgesic effect.

In the control, for instance, the analgesic action of morphine lasted not more than 2-2.5 h, whereas after preliminary injection of $MnCl_2$ in doses of 20-30 μg it lasted up to 4 h. The problem of heterogeneity of opiate receptors is currently under wide discussion in the literature [8, 9]. Receptors specifically binding morphine with high affinity belong to the μ-type. At the same time, it has been shown that during binding of ^{3}H-DADL with a rat brain membrane preparation, at least two types of binding sites take part — with high and low affinity [2]. DADL receptors with high affinity belong to the δ-type [8, 9]. The present writers have studied the effect of cations of metals on affinity of morphine for μ-receptors and the affinity of ^{3}H-DADL for high- and low-affinity types of binding sites *in vitro*. They found [2] that the ability of salts of metals to increase affinity of opioid ligands for corresponding receptors is realized through interaction of the cation of the metal (Me^{n+}) with a specific site of the opiate receptor (Q), in accordance with the scheme $Me^{n+} + Q \leftrightarrows Q^{K_{Me}} \cdot Me^{n+}$, and it is characterized by an equilibrium constant with the value of $K_{Me} = [Q][Me]/[QMe]$. The corresponding values of K_{Me}:K_{Me_1} for receptors of μ-type, K_{Me_2} for high-affinity DADL receptors (δ-receptors), and K_{Me_3} for low-affinity DADL receptors, are given in Table 2. It follows from Table 2 that the value of ED_{50} for salts of different metals, calculated in experiments *in vivo*, change proportionally to values of dissociation constants (K_d) of complexes of cations of these metals with μ-receptors (K_{Me}), and also K_d for complexes of cations with low-affinity DADL receptors (K_{Me_3}). Meanwhile, in the case of δ-receptors, no such proportionality is present: Ni^{++} cations *in vitro* affect affinity of DADL for the δ-receptor much less strongly than Mn^{++} cations, whereas La^{+++} cations, on the contrary, have a stronger activating action than Mn^{++}. The effect of lanthanum salts on the affinity of morphine in experiments *in vitro* is much lower than that of the other metals investigated in the present study. After injection of subtoxic doses of lanthanum salts *in vivo* no significant potentiating effect could be observed. All these findings suggest that one possible mechanism of the potentiating action of the salts of certain metals on morphine-induced analgesia incorporates strengthening of the affinity of morphine for μ-receptors in the brain. The similar characteristics of the effect of salts of metals on affinity of morphine for μ-receptors and for low-affinity binding sites suggests that the low-affinity DADL receptor is a receptor of μ-type and that the analgesic effect of DADL *in vivo* is connected with interaction of this ligand with μ-receptors.

LITERATURE CITED

1. V. E. Gmurman, Probability Theory and Mathematical Statistics [in Russian], Moscow (1972), p. 344.
2. N. V. Porodenko, S. V. Zaitsev, and S. D. Varfolomeev, Bioorg. Khim., 10, No. 7, 902 (1984).
3. R. A. Harris, H. H. Loh, and E. L. Way, J. Pharmacol. Exp. Ther., 196, 288 (1976).
4. G. A. Ferguson, Statistical Analysis in Psychology and Education, New York (1971), p. 324.
5. E. T. Iwamoto, R. A. Harris, H. H. Loh, et al., J. Pharm. Exp. Ther., 206, 46 (1978).

6. J. P. Rosenfeld and M. Hammer, Brain Res., 268, 189 (1983).
7. H. Ueda, H. Amano, and H. Shiomi, Eur. J. Pharmacol., 56, 265 (1979).
8. K. J. Chang and E. Hazum, Proc. Natl. Acad. Sci. USA, 78, 4141 (1981).
9. H. W. Kosterlitz, S. T. Paterson, and L. E. Robson, Br. J. Pharmacol., 73, 939 (1981).

EFFECT OF CAFFEINE ON Ca^{++}-TRANSPORTING FUNCTION OF VESICLES OF THE RAT MYOCARDIAL SARCOPLASMIC RETICULUM

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Caffeine (1,3,7-trimethylxanthine) is a well known potentiator of contraction of skeletal muscle fibers [5]. A study of the mechanism of action of caffeine has shown that this compound can increase the concentration of ionized Ca^{++} in the myoplasm, by liberating Ca^{++} ions from cisterns of the sarcoplasmic reticulum (SR) [6, 7], and reducing the efficiency of active transport of Ca^{++}-dependent ATPase [1]. An important distinguishing feature of the effect of caffeine is its specificity. It has been shown on isolated SR vesicles that caffeine can inhibit the Ca^{++}-transporting function of an SR fraction enriched with terminal cisterns, but not to affect under these circumstances the fraction of elongated tubules [2]. This suggests that caffeine activates the physiological system for liberating Ca^{++} ions from SR, which is considered to be located in the terminal cisterns. The study of the mechanism of action of caffeine on SR can thus shed light on the mechanism of release of Ca^{++} ions in response to excitation. The specific action of caffeine on the Ca^{++}-transporting function of the terminal cisterns enables it to be used to detect fragments of terminal cisterns in the microsomal fraction from homogenate of muscle tissue, myocardial tissue, for example, in which the microsomal fraction contains relatively large numbers of SR fragments, which makes their morphological identification difficult.

The aim of this investigation was to discover the effect of caffeine on active transport of Ca^{++} by different fractions of SR from the rat myocardium and to demonstrate the specificity of its action.

EXPERIMENTAL METHOD

To obtain microsomal fractions, rat hearts were homogenized on a "Polytron" homogenizer (PT-20 generator) in isolation medium containing 0.3 M sucrose, 5 mM sodium azide, and 10 mM histidine (pH 7.0, at 4°C). The ratio of wet weight of tissue to volume of medium was about 1:4. The homogenate was centrifuged for 20 min at 4300g (the bottom of the tube). The supernatant was recentrifuged at the same speed. Heavy (20 min, 14,000 g) and light (30 min, 48,300 g) fractions of microsomes were sedimented successively from the supernatant. Membrane fractions were washed to remove any contamination with actomyosin by suspending them in medium containing 0.6 M KCl, 0.3 M sucrose, and 10 mM histidine (pH 7.0), after which they were sedimented at 48,300g for 30 min. The residues were suspended in 0.3 M sucrose and 10 mM histidine (pH 7.0) and frozen in liquid nitrogen.

Ca^{++} transport was measured by means of a Ca^{++}-selective electrode (Orion 93-20) in medium containing 100 mM KCl, 15 mM potassium oxalate, 5 mM $MgCl_2$, 2 mM ATP, 5 mM NaN_3, SR protein 20-30 μg/ml, and 10 mM HEPES (pH 6.95, at 37°C). Ca^{++} transport was measured by a nephelometric method on an SP-850 spectrofluorometer in a constant-temperature cuvette, equipped

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