DIFFERENCES IN CHARACTERISTICS OF OPIATE AND CATECHOLAMINERGIC RECEPTORS OF STRIATUM AND CEREBRAL CORTEX OF FISHER-344 AND WAG/GSto RATS MAY DETERMINE DIFFERENCES IN POSITIVE REINFORCING ACTION OF MORPHINE

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UDC 615.212.7:547.943].015.4:
616.831.31].076.9

KEY WORDS: morphine; opiate and catecholaminergic receptors; F-344 and WAG/GSto strains of rats

For mental and physical dependence on narcotics to be formed, different individuals require different amounts and different frequencies of consumption of the addicting substance [8, 10, 12]. This may perhaps be connected with differences in perception of the reinforcing action of narcotics.

There is some information that morphine can bind with certain DNA sites [2, 6], can modify expression of the genes of some brain-specific proteins [11], and on the other hand, expression of certain genes in nerve tissue can change the properties of the endogenous opioid system [3, 7]. Thus individual genetic differences in the population may determine resistance or predisposition to the development of dependence on opiates.

The existence of specific binding sites for morphine on brain neuron membranes, and also the abundant information on mediation of the action of narcotics through neurotransmitter processes [1, 5, 13] suggest that genetic differences connected with the pattern of formation of drug dependence, may be manifested first as quantitative and, possibly, also of qualitative differences in expression of membrane receptor proteins in the brain.

The aim of the present investigation was to discover correlation between genetic features of μ-opioid, serotonin-, dopamine-, and adrenergic receptors in the cerebral cortex and striatum of two strains of rats, with their reception of the positive reinforcing action of morphine.

EXPERIMENTAL METHOD

Experiments were carried out on 30 Fisher-344 (F-344) and 28 Wistar albino Glaxo (WAG/GSto) male rats, initially weighing 180-200 g.

Before the experiments began the rats were kept in communal cages with standard lighting and at a constant temperature, with free access to food and water.

Radioligand Investigation of Receptor Binding. Two groups of rats (F-344 and WAG/G), with eight rats in each group, were used. After decapitation the brain was quickly removed and transferred into ice-cold isotonic NaCl solution. Isolated brain structures (cortex, striatum) were frozen in liquid nitrogen and kept until the experiments at −70°C. On the day of the experiments, cerebral cortical tissue was homogenized in 10 volumes of 0.32 M sucrose, whereas the striatum was homogenized in Tris-HCl-buffer, pH 7.7, using a Teflon-glass homogenizer, followed by destruction on a Politron apparatus (position 9.3 · 10 sec). The cerebral cortical homogenate was centrifuged at 2500*g* for 10 min. The supernatant was recentrifuged at 20,000*g* (30 min). The last procedure was repeated another

Laboratory of Experimental Pharmacotherapy of Drug Addiction, Institute for Medico-Biological Problems of Drug Addiction, Government Research Center for the Study of Drug Addiction, Ministry of Health of Russia, Moscow. (Presented by Academician of the Russian Academy of Medical Sciences P. V. Sergeev.) Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 114, No. 9, pp. 296-298, September, 1992. Original article submitted January 22, 1992.

 0007-4888/92/0009-1328$12.50

TABLE 1. Comparison of α_1 and α_2-Adrenergic, S_2-Serotoninergic, D_2-Dopaminergic, and μ-Opiate Receptors of Brain Synaptic Membranes of WAG/G and F-344 Rats (M ± m)

Receptors	B_{max}, fmoles/mg		K_d, nM	
	WAG/G	F-344	WAG/G	F-344
		Cerebral cortex		
μ-Opiate	151+9	132+12*	0,49+0,07	0,64+0,23
α_1-Adrenergic	284+10	264+28	0,31+0,06	0,36+0,07
α_2-Adrenergic	110+17	83+13*	0,43+0,16	0,64+0,13
S_2-Serotonin	346+29	315+40	1,63+0,45	0,91+0,37*
		Striatum		
D_2-Dopamine	525+76	495+40	0,58+0,1	0,32+0,23*

Legend. *$p < 0.01$.

four times. The striatal homogenate was centrifuged at 20,000*g* (30 min), and the residue was resuspended and then centrifuged, this procedure also being repeated four times. The incubation mixture consisted of 200 μl of suspension of the coarse fraction of synaptic membranes and 100 μl of a solution of the isotope in appropriate concentration and, in the case of determination of nonspecific binding, of displacing agents (10^{-6} M). The following were used: ^{3}H-naloxone (61 Ci/mmole), ^{3}H-spiperone (88 Ci/mmole), ^{3}H-clonidine (23.2 Ci/mmole) in concentrations of 0.2-2.0 nM, ^{3}H-prazosin (83 Ci/mmole), and ^{3}H-spiperone (88 Ci/mmole) in concentration of 0.1-1.0 nM. The displacing agents were: morphine, ritanserin, clonidine, prazosin, and haloperidol respectively. Incubation was carried out for 30 min at 25°C. The reaction was stopped by the addition of ice-cold 10 mM Tris-HCl buffer to the incubation mixture, followed by a rapid filtration through glass-fiber GF/F filters and seven washings with the same buffer on an "Automash-2000" harvester (Dynatech). Radioactivity was counted on an "Intertechnique SL-3000" scintillation counter. The protein concentration in the samples was determined by Peterson's method. Concentrations of receptors and dissociation constants were calculated by Scatchard plot, followed by statistical analysis of the data using "hypothesis test for means."

Investigation of Oral Consumption of Morphine Solution by Rats. No water was given for 48 h to 17 F-344 and 15 WAG/G rats, after which they were transferred to individual cages twice a day for 18 days with an interval of 8 h, and for a period of 1 h with drinking bowls containing morphine solution, which the animals could drink freely. For 11 days the rats received liquid only in the form of morphine hydrochloride solutions of increasing concentration (from 0.2 to 0.45 mg/ml). On the 12th day simultaneously with the drinking bowl containing 0.45 mg/ml of morphine solution, the animals also were given access to a bowl containing 0.05 mg/ml quinine (the two bottle method), after which the quantity consumed under conditions of free choice between morphine solution and (or) quinine solution, was recorded individually for 6 days.

Investigation of Conduct of Intravenous Self-Administration of Morphine Solution. In the first stage of the experiment five F-344 and five WAG/G rats were deprived of food for 24 h and then taught to press a pedal fixed in the wall of the experimental chamber ("Gerbrands," USA). Each fifth pressing on the pedal was accompanied by automatic supplying of a food granule (45 mg; from "Bio-Serv," USA). Pressing the pedal produced a result only when carried out when a light shone on the pedal (the light was automatically switched off for 15 sec immediately after reinforcement had been received). After consolidation of the skill, usually on the 7th-10th day of training, PVC catheters were inserted into the subclavian vein of the rats under pentobarbital anesthesia. On the 4th day after the operation the satiated animals, in the same chambers, after the fifth pressing of the pedal, instead of food reinforcement, received 10 μl of morphine solution through a catheter by means of an injector ("Sage Instruments," USA), connected to the modular relay system for automatic injection and recording of data ("Gerbrands," USA). In the first 4 days a dose of 0.5 mg/kg was given, rising to 0.75 mg/kg during the next 4 days and 1 mg/kg during the last 4 days. During the final 4 days of the experiment, instead of morphine solution the system was filled with isotonic NaCl solution. The number of injections received in the course of 30 min, the result of unrewarded presses on the pedal during the automatic injections and during the next interval of 15 sec, marked by absence of light on the pedal, were

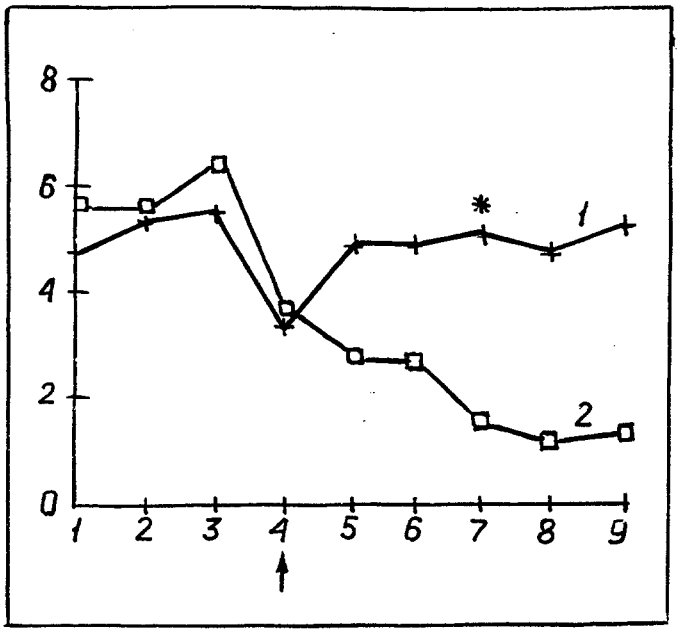

Fig. 1. Consumption of morphine hydrochloride solution (0.45 mg/ml) by rats of test lines before and after allowing animals choice between equally tasty solutions of morphine and quinine. Abscissa, days; ordinate, morphine content (in mg/ml). 1) Fisher-344 rats, 2) WAG/GSto rats; arrow indicates time when animals were allowed to choose between two solutions. *$p < 0.01$.

recorded. The results of the last two series of experiments were subjected to statistical analysis by Student's test for small samples.

EXPERIMENTAL RESULTS

Characteristics of Cerebral Cortical Receptors. Significant differences were found in the characteristics of receptor binding in neuron membranes of the cerebral cortex of F-344 and WAG/G rats. The concentration of μ-opiate and α_2-adrenoreceptors of the synaptic membranes of the neurons was significantly higher in WAG/G rats than in F-344. The sensitivity of the serotonin and dopamine receptors was significantly lower in WAG/G than in F-344 rats. As Table 1 shows, B_{max} for μ-opiate and α_2-adrenoreceptors was significantly higher, whereas K_d for serotonin and dopamine receptors was lower in WAG/G rats than in F-344 rats.

Voluntary Consumption of Morphine Solution during Drinking. Rats of the two different lines were found to utilized morphine solution unequally. Starting with the second day on which the animal could choose between the two liquids, the F-344 rats mainly continued to drink morphine solution ($p < 0.04$) whereas WAG/G rats (starting with the 4th day) drank less of it, preferring to take their liquid requirement in the form of quinine solution (Fig. 1).

Intravenous Self-Administration of Morphine Solution. A significant difference was found in the number of self-injection reactions of morphine in F-344 and WAG/G rats. Daily self-injection of morphine by F-344 rats was 4-10 times greater than the quantity of morphine into WAG/G rats (Fig. 2). Comparison of the number of presses on the pedal to obtain morphine solution and the number of presses leading to injections of isotonic NaCl solution showed that in F-344 rats self-administration behavior was sharply depressed during injection of NaCl solution. In WAG/G rats, on the other hand, there was a tendency for the number of presses to increase (Fig. 3).

The experimental results show differences in perception of the reinforcing action of morphine between F-344 and WAG/G rats. Only in F-344 rats was self-administration strongly inhibited when the morphine solution was replaced by NaCl solution. WAG/G rats evidently did not perceive the positive reinforcing effect of morphine. One

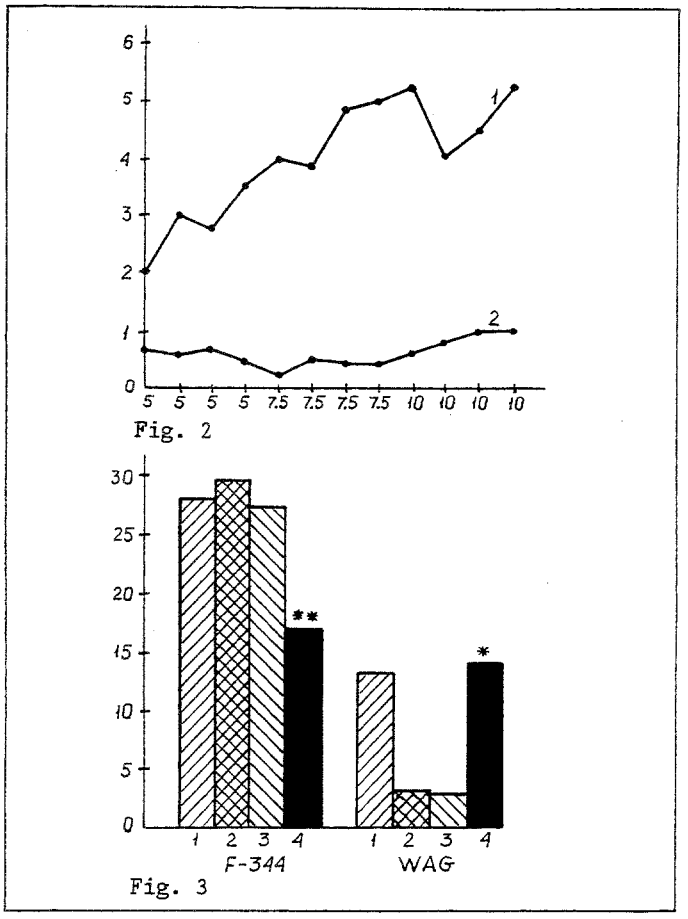

Fig. 2. Intravenous self-administration of morphine hydrochloride solution by rats during 12 days. Abscissa, days; numbers indicate daily concentrations of morphine solution (from 5 to 10 mg/ml) used during self-administration; ordinate, morphine concentration (in mg/kg). Remainder of legend as to Fig. 1.

Fig. 3. Number of responses of pressing pedal by rats during each session (30 min) to obtain intravenous injections of morphine solution of different concentrations and isotonic NaCl solution (during last 4 days as control). Ordinate, number of presses on pedal. Concentration of morphine solution: 1) 5 mg/ml, 2) 7.5 mg/ml, 3) 10 mg/ml; 4) 0.9% NaCl solution. *$p < 0.05$, **$p < 0.01$ compared with days when concentration of morphine solution or self-injection was 7.5 and 10 mg/ml.

of the decisive factors in the absence of reinforcing action of opiates, which we found previously in certain species of animals [9], is the genetically determined pattern of receptor binding of both endogenous opioids and neurotransmitters on brain membranes. However, correlation between the intensity of the positive reinforcing action of morphine and characteristics of receptor interaction in the cerebral cortex, which we and also Elmer and co-workers [4] found, does not rule out the presence of other neurochemical and genetic mechanisms of resistance to the formation of mental dependence on opiates.

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