Effect of Kinurenic Acid on the Acquisition of Intravenous Morphine Self-Administration Habit by Rats

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Male Wistar rats with a silicone cannula implanted into a jugular vein were each placed, for 6 h daily on 5 successive days, in a standard Skinner box where they were trained to self-administer morphine intravenously by pressing a bar. Kynurenic acid, an endogenous nonselective antagonist of excitatory amino acids, injected into rats before their placement in the Skinner box inhibited in a dose-dependent manner the acquisition by them of the morphone self-administration habit, as assessed by the number of times the bar was pressed.

Key Words: *antagonists of receptors for excitatory amino acids; morphine; intravenous drug self-administration*

The brain's "reward" system is a substrate for the reinforcing action of various addiction-producing substances [2,6]. Most narcotics (e.g., opiates, psychostimulants, nicotine) intensify dopamine metabolism in the mesolimbic dopaminergic system [13]. Structures of this system, such as the contiguous nucleus of the septum and the ventral tegmental region, receive large numbers of glutamatergic afferents from the cortex, amygdala, and hippocampus [4,5]. It is only natural, therefore, that neuronal activity in those structures is under glutamatergic control.

Behavioral experiments have shown that systemically injected antagonists of excitatory amino acids (EAA) attenuate the reinforcing effect from stimulation of the reward system with pharmacological agents (opiates) or electrical pulses (brain self-stimulation response) [1]. Antagonists of NMDA-subtype glutamate receptors weakened the stimulatory and reinforcing properties of a number of narcotics on local administration into the contiguous nucleus [10,11].

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The purpose of the present experiment was to evaluate the effect of kynurenic acid, a nonselective EAA antagonist, on the acquisition of the intravenous morphine self-administration habit by rats.

MATERIALS AND METHODS

Male Wistar rats (body weight 220-240 g) from the Rappolovo Nursery were used. Primary reinforcing properties of morphine were investigated using the intravenous self-administration procedure described by Ramsey and van Ree [12]. Seven days before the experiment, a silicone cannula (1.26 mm in outside diameter) was implanted into the right jugular vein of each rat. On day 6 postimplantation rats were placed in a room with an inverted lighting regimen (the light period from 20:00 to 08:00) where they had limited access to food so as to reduce their body weight to 80% of its initial value.

All testing was done in standard Skinner boxes, each with two bars ("rewarding" and "nonrewarding") on one side. Pressing the "rewarding" bar by the rat actuated a computer-controlled microinjection pump with the result that the animal received

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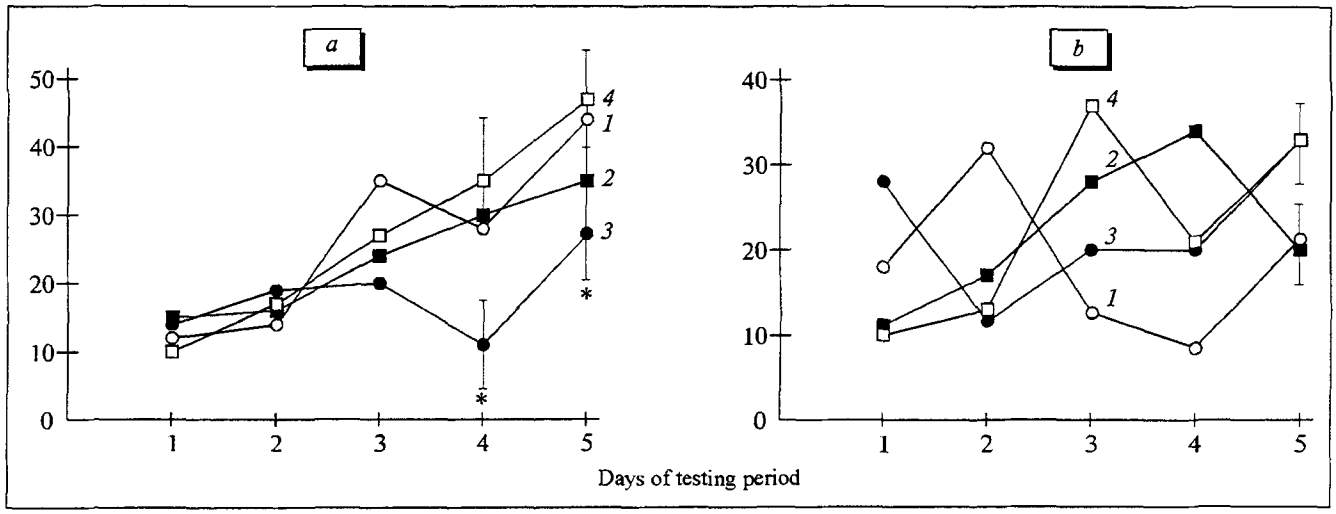

Fig. 1. Effect of kynurenic acid on the acquisition of morphine self-administration response by rats. Ordinate: number of presses of the reinforced ("rewarding") (*a*) and nonreinforced ("nonrewarding") (*b*) bars over 360 min. Kynurenic acid in doses of 50 (*1*), 100 (*2*), and 150 (*3*) mg/kg or its solvent (*4*) were injected 1 min before placing the rat in the Skinner box. *$p<0.05$ (by Newman-Kelse's test); there were 7-12 rats per group.

a single intravenous infusion of 0.25 ml of morphine solution (0.128 mg morphine per ml of isotonic NaCl solution; approximately 0.16 mg/kg body weight) or of isotonic NaCl solution. On 5 successive days, each rat was placed in a Skinner box for 360 min immediately after receiving kynurenic acid (RBI) (50-150 mg/kg intraperitoneally) or its solvent (2% Tween-80, 1 ml/kg). The pH of the solutions was adjusted to 7.4 with 0.1 and 0.01 M NaOH and HCl solutions.

Rats were "prompted" to the instrumental responses (bar presses) by being given two compulsory infusions each day at the beginning of testing in the Skinner box and two additional infusions on day 1 every 60 min of the 360-min testing session. Whenever a rat had received 60 infusions during a single session before its end, the test was discontinued. Over the 5-day testing period, the number of presses of each bar was recorded.

The results were statistically treated by one-way analysis of variance (ANOVA) with *post hoc* application of Newman—Kelse's test.

RESULTS

As can be seen in Fig. 1, rats given the solvent instead of kynurenic before daily sessions in the Skinner box acquired the habit of intravenous morphine self-administration. The one-way ANOVA demonstrated a significant effect of the testing period (5 successive days) on the incidence of reinforced bar presses ($F_{(4.37)}=5.89$, $p<0.05$), but not on that of nonreinforced bar presses ($F_{(4.37)}=0.78$, $p>0.05$). In the control tests, where the morphine solvent (isotonic NaCl solution) was substituted for the morphine solution (data not shown), no significant differences between the rates of pressing the reinforced and nonreinforced bars were noted over the 5-day testing period ($F_{(4.33)}=0.94$, $p>0.05$ and $F_{(4.33)}=1.12$, $p>0.05$, respectively).

Rats preinjected with kynurenic acid at 150 mg/kg (but not those preinjected with it at 50 or 100 mg/kg) showed no significant increase in the incidence of morphine reinforced or nonreinforced bar presses over the testing period ($F_{(4.76)}=1.24$, $p>0.05$ and $F_{(4.76)}=2.04$, $p>0.05$, respectively). On days 4 and 5, however, the incidence of reinforced bar presses in this group was significantly lower than in the control group preinjected with the solvent instead of kynurenic acid ($p<0.05$ by Newman—Kelse's test).

The nonselective EAA antagonist kynurenic acid thus prevented the rats from acquiring the habit of intravenous morphine self-administration. This acid is an endogenous ligand of EAA receptors [15], and the elevation of its level in the body through systemic administration produces effects that are physiological in terms of the pharmacological action range. It may be stated, therefore, that the results of this study indicate that EAA are involved in mediating the natural regulation of the reward system.

The mesolimbic dopaminergic system is regarded as the possible substrate for the action of most narcotics [2,6], although there is evidence that opiate dependence may be dopamine-independent [3, 9]. Moreover, injecting antagonists of NMDA-subtype glutamate receptors into the septal contiguous nucleus of rats pretrained to self-administer narcotics intravenously was found to result in diminished

intake of cocaine but not of heroine [11]. It should be noted that in the present experiment we studied self-administration of morphine (not heroin) by untrained rats. The seeming discrepancy between the results can therefore be explained by procedural differences which may be very substantial in the case of drug self-administation by the intravenous route [8]. In addition, the septal contiguous nucleus is a nonhomogeneous structure as regards both the distribution of NMDA- and non-NMDA-receptor subtypes [7] and the significance of its various subterritories for the process of narcotic self-administration [14]. It should be emphasized in this connection that kynurenic acid is a nonselective antagonist of NMDA- as well as non-NMDA-receptor subtypes [15].

Since EAA receptor antagonists have been previously shown to attentuate the reinforcing action of opiates in tests for brain self-electrostimulation and conditioned place-preference response and to delay the onset of opiate tolerance and physical dependence [1], the findings from the present study may be found useful in the development of improved methods for the prevention or treatment of opiate dependence.

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