EFFECTS OF ELECTRICAL STIMULATION OF NUCLEUS RAPHE DORSALIS ON INITIATION OF MORPHINE SELF-ADMINISTRATION IN RATS

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SUMMARY: Recent neurochemical studies suggest that alterations in functions of the central neurotransmitters may be involved in addiction. Serotonin (5-HT), dopamine (DA) and endogenous opiate are the most important neurotransmitters involved in addiction. Nucleus raphe dorsalis (NRD) on the other hand is the important site that secrete or modulate these substances. Release of 5-HT can be evoked by focal electrical stimulation of NRD. In this study electrical stimulation (30 min: pulse 0.5 ms, 150 μA, 20 Hz) of the antero-dorsal part of the nucleus raphe dorsalis produced significant decrease in initiation of morphine self-administration during 10 consecutive 2-h sessions. During all sessions lever-pressing behavior was measured. After the last test session, morphine withdrawal syndrome signs (wet dog shakes, jumping, writhing and diarrhea) in the naloxone-induced behavior, were measured. The results show that these withdrawal syndrome signs are decreased by application of electrical stimulation of NRD. It is concluded that electrical stimulation of NRD enhances the serotonergic and endogenous opiate transmission, and this activation might be involved in reduction of craving for morphine.

Key Words: Raphe nucleus, electrical stimulation, morphine sulfate, dependence.

INTRODUCTION

Nucleus raphe dorsalis (NRD) is one of the specific areas in the brain which is implicated in the loss of control inherent in the addictive use of multiple drugs such as morphine (1-3). This site contains cell bodies and receptors for serotonin and endogenous opiate respectively, where multiple drugs and morphine can often produce their effects. Electrical stimulation of the midbrain raphe nucleus has proved to be an effective means to increase axonal 5-HT release and turnover in the forebrain and hypothalamus (4-6). The NRD is a focal point in ascending and descending modulation of addiction at which (their modulation) can be induced by focal electrical stimulation (7-9). Furthermore, it has been shown that the 5-HT is involved in the mechanisms, leading to the withdrawal syndrome precipitated by naloxone in the rat (10-12). Biological causes of drug addiction and the co-morbid psychiatric disorders have been repeatedly proposed and it has also been suggested that the same changes in central neurotransmitter functions, in particular

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the serotonergic system, may be involved in the pathogenesis of the combination of two types of disorders (13-15).

The present study has been designed to investigate the role of the electrical stimulation of NRD on drug reward during initiation of drug self-administration and particularly in its motivational aspects of drug-taking behavior. Recently a behavioral paradigm has been investigated which can assess the motivation aspect of initiation of drug self-administration (16,17): i.v. Self-administer a drug during ten consecutive sessions by pressing one out of two levers present in the test cage. During the 30 min, preceding the self-administration sessions, the animals receive the electrical stimulation and then have access to both levers, but only pressing the reinforcement lever results in a dose of drug infusion. During the session, the numbers of self-infusion and lever-pressing were recorded.

MATERIALS AND METHODS

Animal and housing conditions

Animals were male Wistar rats bred from our own live stock weighing 210-270 g at the time of surgery. Before surgery, the animals were group-housed, received food and water *ad libitum* and were maintained under a day/night cycle with lights on between 07.00 a.m. and 07.00 p.m. After surgery, the animals were placed in individual home cages and were allowed to recover from the operation for 5-7 days before testing was started. During testing food and water were available in the home cages. The day/night cycle was reversed 3 days before testing, so that the animals were tested during the dark phase of the cycle.

Surgery (cannula and electrode placement)

Animals were anesthetized with Ketamine (15 mg/kg) and Rampon (0.1 mg/kg) and a cannula was inserted into the jugular vein (18). The cannula was guided subcutaneously up to the skull where it was fixed to a curved metal tube, which was secured onto the skull with screws. In the same surgery session the animals were prepared for electrode placement. Stimulating electrodes (bipolar coaxial stainless steel, active length 900 μm: SNEX100, Roucaire, France) were lowered into the NRD according to the Paxinos and Watson (19) atlas (AP+1.2, L-1.6, V+7.4) then fixed with dental acrylic cement.

Electrical stimulation

Stimulation was applied with a stimulator (type 2521) device and the current delivered through the set-up was checked with an oscilloscope (8203 SAIRAN). Parameters of stimulation were monophasic pulses of 0.5 ms duration, 150 μA current, 20 Hz. Stimulation was applied for 30 min before starting the self-administration sessions.

Procedure

Details of the procedure have been recorded previously (16, 20). Testing was done in standard operant conditioning cages placed in sound-attenuated rooms. The test cages were equipped with two levers, one that was marked by the red light placed just above the lever. The i.v. cannula of the animals were connected to an infusion pump. Depression of the lever marked by the red light (reinforcement lever; RL) resulted in an i.v. infusion of 0.20 ml fluid during 6 s on a continuous reinforcement schedule. The fluid was saline in sham group and morphine sulfate (TEMAD IRAN) with 5 mg/ml concentration in other groups. The red light went off during the infusion and pressing the lever during this time did not result in an infusion action. Depression of the other lever (non-reinforcement lever; i.v. self-administer a drug solution for 3 hours a day (starting at 10.00 a.m.) or until a maximum of 60 self-infusion was reached. Testing took place on ten consecutive daily sessions. Lever-press behavior was measured during drug self-administration sessions.

Withdrawal syndrome sign

The abstinence syndrome was precipitated with an intraperitoneal injection of 2.0 mg/kg naloxone HCL, dissolved in saline. Withdrawal signs within 30 min were precipitated by naloxone in all experimental groups consisted mainly of wet dog shakes, jumping, writhing and diarrhea.

Localization of stimulating electrode

At the end of each experiment, the brain was removed and the final position of the stimulating electrode was determined by inspection of Cresil Violet stained brain section. Data were included in the final analysis only if the final position of the electrode tip was found to be in the region of the NRD, as expected.

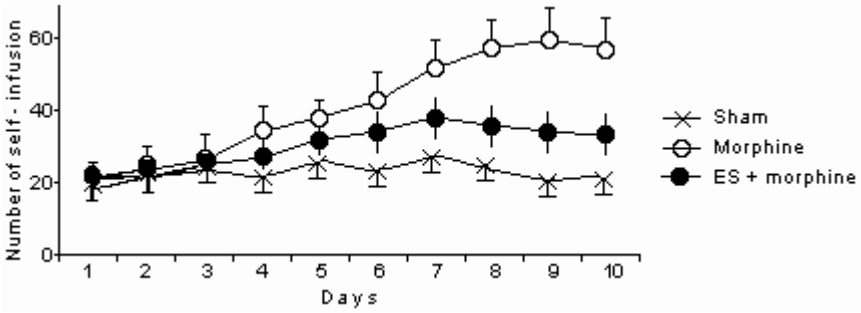
Experimental design

In separated experiments groups of animals (n=6-8) were electrode and cannula implanted, divided to three groups: a) sham group did not receive electrical stimulation nor morphine in self-administration sessions, b) control group did not receive electrical stimulation, were given only morphine in self-administration sessions, c) active group received both electrical stimulation before sessions and morphine in self-administration sessions.

Statistical analysis

Data are presented as the mean±SEM. The data obtained during self-administration sessions were

Figure 1: Effect of electrical stimulation of NRD on initiation of drug self-administration. Animals were tested during 10 consecutive daily in 3 h sessions. The mean number of self-infusions is plotted versus the day of testing in three groups: sham which did not receive electrical stimulation nor morphine in self-administration sessions, control group which received morphine but not electrical stimulation in self-administration sessions, active group (ES+Morphine) which received both electrical stimulation before sessions and morphine in self-administration sessions.



analyzed per unit dose experiment using two way analysis of variance with repeated measurements (MANOVA). Stimulation (vs. sham) and time (ten sessions) were grouping variables and number of self-infusion (SI), number of responses on the reinforcement lever (RL) or number of responses on the non-reinforcement lever (NRL) the dependent variable. The difference in total number of SI (summed over ten sessions) between activated and non-activated animals were analyzed using student's t-test.

RESULTS

Initiation of drug self-administration

The number of self-infusions (SI) during initiation of morphine self-administration is presented in Figure 1. The electrical stimulation of NRD resulted in a significant reduction in number of SI ($p<0.01$) in comparison with morphine group. The number of SI in the activated group was less than that of the morphine group and more than sham group ($p<0.01$).

Responding on the reinforcement lever (RL) significantly decreased ($p<0.01$) in the activated group (Figure 2). Responding on non-reinforcement lever (NRL) did not reveal any significant effect between morphine and activated group (Figure 2).

Withdrawal syndrome signs

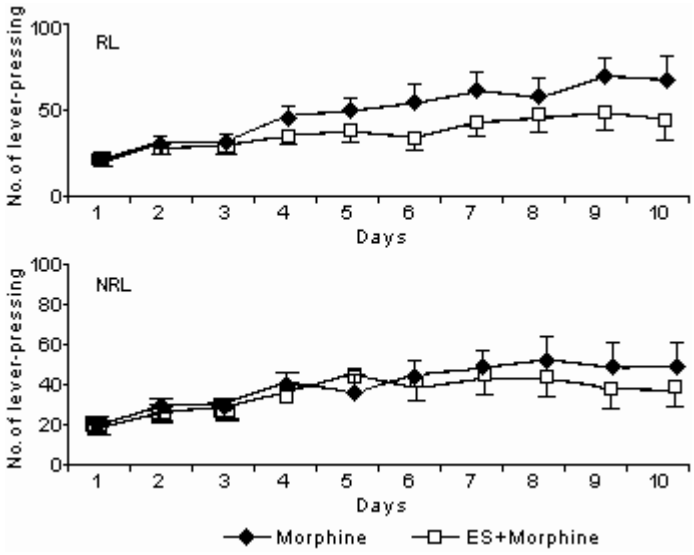
Table 1 shows the effect of electrical stimulation of NRD on withdrawal syndrome signs included wet dog shakes, jumping, writhing and diarrhea. These signs were recorded during 30 min after naloxone injection (2 mg/kg i.p.). All of the signs were reduced in the activated group in comparison with control group (Table 1).

Table 1: Comparison between morphine withdrawal syndrome signs included wet dog shakes, jumping, writhing and diarrhea during 30 minutes after naloxone injection (2 mg/kg i.p.). Data are represented as mean±SE in sham, control group (morphine) which did not receive electrical stimulation and only received morphine in self-administration sessions and active group (ES+morphine) which received electrical stimulation before sessions and morphine in self-administration sessions.

Withdrawal signs / Groups	Wet dog shakes (mean±SE)	Jumping (mean±SE)	Writhing (mean±SE)	Diarrhea (mean±SE)
Sham	0	2.4 ± 0.45	2.8 ± 0.95	0
Morphine	30.2 ± 3.6	18.2 ± 2.3	28.2 ± 3.1	++++
ES+morphine	18.24 ± 1.9**	8.6 ± 0.92**	21.2 ± 2.6*	++

*p<0.05, **p<0.01

Figure 2: Effect of electrical stimulation of NRD on responding on the reinforcement lever (RL) and non-reinforcement lever (NRL) during i.v. morphine self-administration. Animals were allowed to self-administer during 10 consecutive daily in 3 h sessions. The mean number of responses on RL and NRL is plotted versus the day of testing in control group which did not receive electrical stimulation but received only morphine in self-administration sessions and active group which received electrical stimulation before sessions and morphine in self-administration sessions.



DISCUSSION

The present study employed a behavioral paradigm in which lever-press behavior of animals can be measured during drug self-administration (17, 20). Behavior during the self-administration sessions is thought to be mainly dependent on the craving for morphine (16,17). Previous studies have shown that electrical stimulation of NRD release 5-HT, suggesting that serotonergic nerve terminals of the dorsal raphe pathway were being activated selectivity. Addiction is a biologically based disease in which biology and behavior interact in complex way. Evidence suggests that abnormalities in several central neurotransmitters, including serotonin (5-HT) and dopamine (DA) may cause addiction (21-23). Serotonergic abnormalities have been hypothethized for most of the major psychiatric disorders (2, 24). It has been shown that augmentation of 5-HT neurotransmission attenuates alcohol consumption whereas depletion enhances it (2,12). Thus, 5-HT may represent a common denominator for various behavior disorders.

In the field of substance abuse several controlled studies of the effects of the focal electrical stimulation have been conducted with some encouraging results. The present study provides evidence that electrical stimulation of NRD affects several concomitants of morphine abuse. Electrical stimulation of NRD appears to have effected almost immediate (Figure 1) after 5 days treatment, this (lever-pressing) response is already significantly reduced. The influence of electrical stimulation of NRD upon withdrawal syndrome signs were significantly reduced the syndrome signs (Table 1). It is worth mentioning that the changes in withdrawal syndrome signs may be caused by reduction of morphine consumption during the self-administration sessions.

For the moment, the only clue as to the mechanisms of action of electrical stimulation of NRD comes from recent studies in animal models which draw attention to the release of serotonin and endogenous opiates (25, 26). Low levels of endorphin, serotonin and dopamine have been observed repeatedly in addiction, prompting some authors to propose an endorphin deficiency hypothesis in drug abuse (25, 27-29). Our preliminary results indicate that electrical stimulation of

NRD might influence these mechanisms. At present we do not know of any associated behavioral changes that may explain these results. Whether the effect of electrical stimulation of NRD on morphine self-administration is primary or secondary to the significant improvement of stress symptoms, mood and in particular depression, is an open question.

REFERENCES

1. Lemarquand D : Serotonine and alcohol intake, abuse and dependence. Clinical Evidence Biol Psychiatry, 36:320-337, 1994.

2. Mcbride WG, Ljubic T : Serotonin mechanisms in alcohol drinking behavior. Drug Der Re, pp 177-183, 1993.

3. Aiello P, Bartolini A, Bartolini R, Galli A : Effects of morphine, physostegmine and raphe nuclei stimulation on 5- HT release from cerebral cortex of the cat. Br J Pharmac, 65:547-555, 1979.

4. Richard JG, Gugenhein R : Serotonergic axons in the brain. Neurosci, 5:14-20, 1982.

5. Liskow BI, Goodwin DW : Pharmacological treatment of morphine intoxication, withdrawal and dependence. A critical review. J Stud Alcohol, 48:356-370, 1987.

6. Hajos M, Sharp T : A 5-HT lesion markedly reduced the incidence of burst-firing dorsal raphe nucleus in the rat. Neurosci Lett, 9:204:161-164, 1995.

7. 14-Qiao JT, Scolnick M, Dafney N : Dorsal raphe and external electrical stimulation modulate noxious input to single neurons in nucleus parafascicularis thalami. Brain Res Bull, 21:671-675, 1998.

8. Padjen AL, Dongier M, Malec T : Effects of cerebral electrical stimulation on alcoholism. Alcoholism: Clinical and Experimental Research, 19:1004-1010, 1995.

9. Patterson MA, Firth J, Gardiner R : Treatment of drug alcohol and nicotine addiction by neuroelectric therapy. J Bioelectricity, 3:193-221, 1984.

10. Heiner B, John R : 5-HT1 receptor agonist attenuate the naloxone-induced jumping behavior in morphine-dependence mice. EJP, 162:19-27, 1989.

11. Wallis CJ, Rezazadeh SM, Lal H : Role of serotonin in ethanol abuse. Drug Dev Res, 30:178-188, 1993.

12. Anil G, Hemendra N : Brain and spinal cord 5-HT2 receptors of morphine-tolerant-dependent and abstinent in rats. EJP, 167:185-192, 1989.

13. Fredrico G, Francisco S, Telmia G : Role of 5-HT in stress, anxiety and depression. Pharmaco Biochem and Behavior, 54:129-141, 1996.

14. Tomkins DM : Effects of 5-HT3 antagonist on ethanol intake Psycho Berl, 117:479, 1985.

15. Sharp T, Bramwell SR, Graham S : Release of endogenous 5-HT in rat hypocampus evoked by electrical stimulation of dorsal raphe nucleus. Neuroscience, 39:629-637, 1990.

16. Stewart J, De Wit H, Eikelboom R : Role of unconditioned and conditioned drug effects in the self-administration of opiates and stimulants. Psychol Rev, 91:251-268, 1984.

17. Glick SD, Cox RD : Changes in morphine self-administration after brain stem lesions in rats. Psychoparmacol, 52:151-156, 1977.

18. Van Ree JM, Slangen JF : Intravenous self-administration of drug in rats. J Pharmacol Exp Ther, 204:547-557, 1978.

19. Paxinos G, Watson C : The rat brain in stereotaxic coordinates. Academic Press, New York, 1998.

20. Zito KA, Vickers G, Robert DCG : Disruption of cocaine and heroin self-administration following kainic acid lesions of the nucleus accumbens. Pharmacol Biochem Behav, 23:1029-1036, 1985.

21. Van Ree JM, Ramsey NF : The dopamine hypothesis of opiate reward challenged. Eur J Pharmacol, 134:239-243, 1987.

22. Fletcher PJ : Effect of combined or separated 5, 7-DHT lesion of the dorsal raphe nuclei on food reinforcement. Brain Res, 27:45-54, 1995.

23. Wise RA, Rompre PP : Brain dopamine and reward. Annu Rev Psychol, 40:191-225, 1989.

24. Lemarquand D, Pihl RO, Benkelfet C : Serotonin and alcohol intake, abuse and dependence. Biol Psychiatry, 36:326-337, 1989.

25. Smart D, Lambret D : The stimulatory effects of opioids and their possible role in the development of tolerance. Tips, 17:264-268, 1996.

26. Tsutomo S, Ykiji F, Miwa M : Enhancement of morphine withdrawal signs in the rat after chronic treatment with naloxone. EJP, 178:239-242, 1990.

27. Extein I, Dackis CA : Brain mechanism in cocaine dependence. In Washton AM, Gold MS, New York, Guilford Press, pp 73-84, 1987.

28. Sweep CGJ, Weigant VM : Beta-Endorphine in brain limbic structures as neurochemical correlate of psychic dependence on drugs. Life Sci, 44:1133-1140, 1989.

29. Anne F : Mechanism of LSD: a glimpse into the serotonergic system. Biology 202, Third web reports, pp 1-3, 1999.

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