ELSEVIER

Behavioural Brain Research 65 (1994) 117–119

BEHAVIOURAL BRAIN RESEARCH

Short communication

The inhibitory effect of norharman on morphine withdrawal syndrome in rats: comparison with ibogaine

Susanne L.T. Cappendijk [a,*], Durk Fekkes [b], Michailo R. Dzoljic [a]

Department of [a] Pharmacology [PO Box 1738] and [b] Psychiatry, Faculty of Medicine and Health Sciences, Erasmus University Rotterdam, 3000 DR Rotterdam, The Netherlands

Received 21 June 1994; revised 5 August 1994; accepted 5 August 1994

Abstract

Norharman (20 mg/kg, i.p.) and ibogaine (40 mg/kg, i.p.) significantly attenuated naloxone (4 mg/kg, i.p.)-precipitated withdrawal syndrome in morphine-dependent rats. Several withdrawal signs, such as teeth-chattering, chewing, penile licking and diarrhoea, were decreased by both norharman and ibogaine. In addition, norharman reduced also the withdrawal grooming and rearing. It is concluded that both norharman and ibogaine are inhibitors of withdrawal syndrome in morphine-dependent rats.

Key words: Ibogaine; Morphine dependence; Morphine withdrawal; Naloxone; Norharman; Rat

Norharman (β-carboline) is an endogenous occurring substance in brain and other tissues in rats and man [13,14]. Recently, elevated plasma levels of norharman were detected in chronic alcoholics [17] and heroin addicts [18]. These data favour an idea of involvement of norharman in drug dependence processes. A structurally related substance to norharman is ibogaine. Both, norharman and ibogaine are indole derivatives with psychogenic/hallucinatory properties [2,9]. It has been shown that ibogaine attenuates morphine withdrawal [8,12] and interrupts drug dependence [5,11]. These facts justify a further elucidation of the effects of these two substances in drug dependence phenomena. In order to make a comparison between these two chemically and behaviorally (psychogenic/hallucinatory) similar substances, we studied the effects of both drugs, norharman and ibogaine on naloxone-precipitated withdrawal in morphine-dependent rats.

Male Wistar rats (TNO Zeist), weighing 290–330 g were housed in groups and had a free access to food and water. The room was maintained on a 12-h light/dark cycle (lights on 08.00 h), with constant temperature (21 °C) and humidity (55%). Morphine dependence was induced by implantation of a morphine base pellet (75 mg/rat, s.c., $n = 30$) on the back of the animal under ether anaesthesia [4]. All morphine-dependent animals were used only once. Morphine-dependent rats were divided into three groups, pretreated intraperitoneally with vehicle (distilled water, $n = 10$), norharman (20 mg/kg, $n = 10$) or ibogaine (40 mg/kg, $n = 10$). The selected doses of norharman and ibogaine are biologically active, shown by previous studies [5,15]. The withdrawal syndrome in morphine-dependent animals was precipitated by naloxone (4 mg/kg, i.p.), given 30 min after vehicle, norharman or ibogaine. The naloxone treatment occurred 72 h following pellet implantation. The observer was 'blind' to the treatment order and registered the withdrawal symptoms during 30 min following injection of naloxone. The withdrawal signs were scored according to the weighting factors described by Neal and Sparber [16]. In short, the signs observed during a mild withdrawal syndrome were assigned with 1 (diarrhoea, chewing, grooming, irritability on touch, rearing), whereas the sign rhinorrhoea, observed during severe withdrawal was assigned a 3. All other withdrawal signs, teeth-chattering, wet-dog shakes, penile licking, ptosis and jumping were assigned by a weighting factor 2.

Norharman (Sigma, England) and ibogaine (Sigma, England) were administered in volume of 2.2 ml/injection. Naloxone HCl (Sigma Chemical Co., St Louis, MO) was given in volume of 1 ml/kg. The pH of drug solutions and vehicle were adjusted to 7–8. All drugs were dissolved in distilled water. Data were evaluated by using the non-parametric Kruskall–Wallis one-way analysis of variance,

* Corresponding author.

SDI 0166-4328(94)00110-3

followed by the Mann–Whitney *U*-test, with a level of $P<0.05$ being considered significant [10].

A decreased locomotion and exploratory behaviour was observed in norharman (20 mg/kg, i.p.)-treated naive ($n=6$) and morphine-dependent ($n=10$) animals. This effect lasted 5–20 min. In contrast, the ibogaine (40 mg/kg, i.p., $n=10$) induced within 4 min tremor and excitatory behaviour (jumping or violent locomotion on touch). The behavioral effects, induced by norharman or ibogaine disappeared within 30 min.

This study is the first demonstration that norharman, a physiological substance significantly attenuates a naloxone-precipitated withdrawal syndrome in rats (Fig. 1A). Ibogaine, similarly to previous data [8,12], also reduced naloxone-precipitated withdrawal syndrome (Fig. 1A). Related to the specific symptoms, both norharman and ibogaine attenuated teeth-chattering, chewing, penile licking and diarrhoea (Fig. 1B,D,F,G). Grooming and rearing response were reduced by norharman only (Fig. 1C,E). These data indicate that norharman and ibogaine induced a similar (but not identical) decrease of opioid withdrawal symptoms.

Although a mechanism of action of norharman and ibogaine is not known, an involvement of the opioid system could be considered, since both drugs interfere with opioid receptors as agonists. Norharman acts as a partial μ-agonist [2], while ibogaine is an agonist of κ-receptors [6]. The binding activity of both drugs to central opioid receptors with possible displacement or preventing the binding of naloxone to opioid receptors may lead to an antiwithdrawal effect. In periphery μ- and κ-agonists can depress the acetylcholine release from the cholinergic neurons of myenteric plexus [3]. This effect may contribute to the decreased intensity of withdrawal diarrhoea, induced by norharman and ibogaine.

The other neurotransmitter system which could be involved in the decreased expression of the opioid withdrawal is the glutaminergic system. Glutamate antagonists may prevent morphine abstinence in mice and guinea pigs [4,19]. Consistent with this finding, morphine is able to block the glutamate-induced excitation in the monkey [20] and in the mouse [1]. The fact that both norharman and ibogaine have also morphine-like properties [2,6] favour a hypothesis that blockade of the glutamate-mediated transmission could contribute to the attenuation of the excitatory character of withdrawal syndrome. This idea has been supported by Dowson [7], showing that harmala alkaloids inhibit the transmission at the glutamate-mediated neurons.

In conclusion, these experiments show that norharman and ibogaine attenuated the opioid withdrawal syndrome and favour an idea of an inhibitory role of both drugs in the expression of morphine abstinence. Although an involvement of the opioid- and/or glutamate-neurotransmitter system could be considered at present as a main underlying mechanism for the attenuation of withdrawal syndrome, the precise mechanisms of action of norharman and ibogaine remain unclear. However, of particular im-

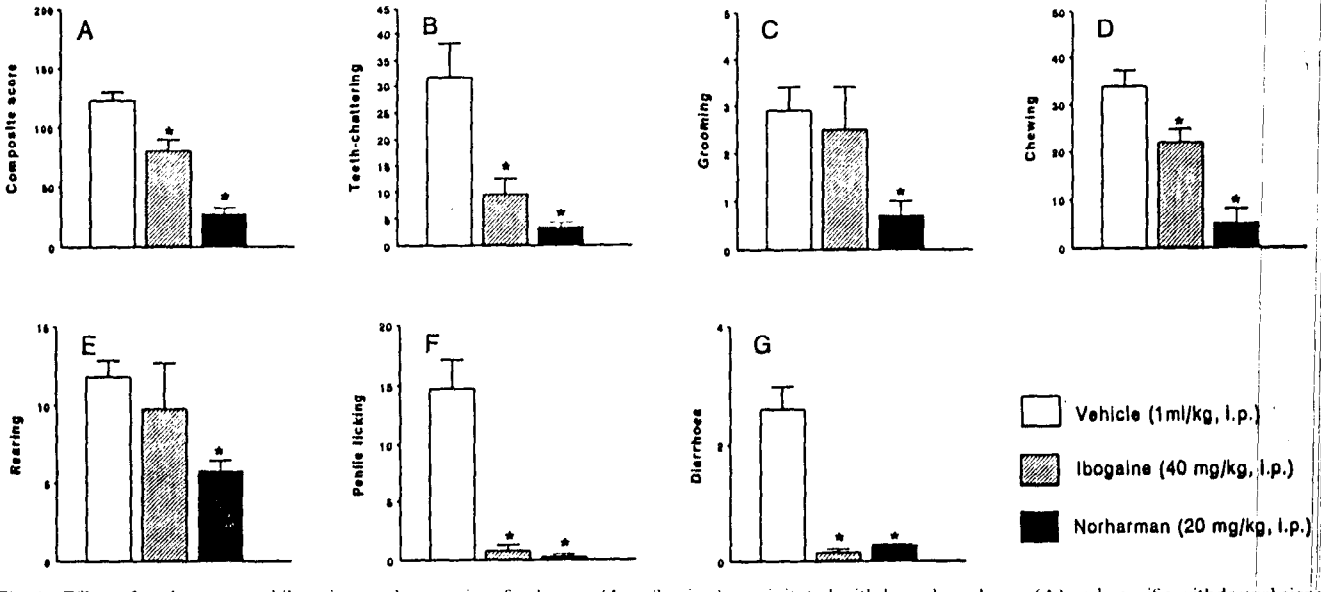

Fig. 1. Effect of norharman and ibogaine on the severity of naloxone (4 mg/kg, i.p.)-precipitated withdrawal syndrome (A) and specific withdrawal signs (B–G) in morphine-dependent rats. Morphine dependence was induced by implantation of morphine pellet on the back of the animal under ether anaesthesia. Animals were treated with vehicle (distilled water, 1 ml/kg, i.p., $n=10$), ibogaine (40 mg/kg, i.p., $n=10$) or norharman (20 mg/kg, i.p., $n=10$), 72 h following pellet implantation and 30 min prior naloxone. Data in Fig. 1A are expressed as composite score, determined by counting the number of all observed withdrawal signs, during the 30-min period of abstinence. The withdrawal signs were scored according to the method described by Neal and Sparber [16]. All data were expressed as mean ± S.E.M. * Significant decrease of withdrawal syndrome or signs (Mann–Whitney *U*-test, $P<0.05$) compared to the control group. Note that norharman and ibogaine attenuated the severity of withdrawal syndrome and frequency of withdrawal signs.

portance would be a further clarification of the role of norharman as a physiological modulator of morphine withdrawal phenomena.

This research was supported by Addiction Research Institute Rotterdam.

[1] Aanonsen, L.M. and Wilcox, G.L., Nociceptive action of excitatory amino acids in the mouse: effects of spinally administered opioids, phencyclidine and *sigma* agonists, *J. Pharmacol. Exp. Ther.*, 243 (1987) 9–19.

[2] Airaksinen, M.M. and Kari, I., β-Carbolines, psychoactive compounds in the mammalian body, *Med. Biol.*, 59 (1981) 190–211.

[3] Burks, T.F., Fox, D.A., Hirning, L.D., Shook, J.E. and Porreca, F., Regulation of gastrointestinal function by multiple opioid receptors, *Life Sci.*, 43 (1988) 2177–2181.

[4] Cappendijk, S.L.T., De Vries, R. and Dzoljic, M.R., Excitatory amino acid receptor antagonists and naloxone-precipitated withdrawal syndrome in morphine-dependent mice, *Eur. Neuropsychopharmacol.*, 3 (1993) 111–116.

[5] Cappendijk, S.L.T. and Dzoljic, M.R., Inhibitory effects of ibogaine on cocaine self-administration in rats, *Eur. J. Pharmacol.*, 241 (1993) 261–265.

[6] Deecher, D.C., Teitler, M., Soderlund, D.M., Bornmann, W.G., Kuehne, M.E. and Glick, S.D., Mechanisms of action of ibogaine and harmaline congeners based on radioligand binding studies, *Brain Res.*, 571 (1992) 242–247.

[7] Dowson, R.J., Clements, A.N. and May T.E., The action of some harmala alkaloids on transmission at a glutamate-mediated synapse, *Neuropharmacology*, 14 (1975) 235–240.

[8] Dzoljic, E.D., Kaplan, C.D. and Dzoljic, M.R., Effect of ibogaine on naloxone-precipitated withdrawal syndrome in chronic morphine-dependent rats, *Arch. Int. Pharmacodyn.*, 294 (1988) 64–70.

[9] Farnsworth, N.R., Hallucinogenic plants, *Science*, 162 (1968) 1086–1092.

[10] Glantz, S.A., *Primer of Biostatistics: Methods Based on Ranks*, McGraw-Hill, New York, NY, 1989, pp. 291–317 pp.

[11] Glick, S.D., Rossman, K., Steindorf, S., Maisonneuve, I.M. and Carlson, J.N., Effects and aftereffects of ibogaine on morphine self-administration in rats, *Eur. J. Pharmacol.*, 195 (1991) 341–345.

[12] Glick, S.D., Rossman, K., Rao, N.C., Maisonneuve, I.M. and Carlson, J.N., Effects of ibogaine on acute signs of morphine withdrawal in rats: independence from tremor, *Neuropharmacology*, 31 (1992) 497–500.

[13] Greiner, B. and Rommelspacher, H., Two metabolic pathways of tetrahydro-norharmane (tetrahydro-beta-carboline) in rats, *Naunyn Schmiedeberg's Arch. Pharmacol.*, 325 (1983) 349–355.

[14] Honecker, H. and Coper, H., Tetrahydronorharmane (THN) and 6-hydroxy-norharmane: physiological components in platelets and urine of man, *Naunyn Schmiedeberg's Arch. Pharmacol.*, 302 (1978) R63.

[15] Morin, A.M., β-Carboline kindling of the benzodiazepine receptor, *Brain Res.*, 321 (1984) 151–154.

[16] Neal, B.S. and Sparber, S.B., Mianserin attenuates naloxone-precipitated withdrawal signs in rats acutely or chronically dependent upon morphine, *J. Pharmacol. Exp. Ther.*, 236 (1986) 157–165.

[17] Rommelspacher, H., Schmidt, L.G. and May, T., Plasma norharman (β-carboline) levels are elevated in chronic alcoholics, *Alcohol Clin. Exp. Res.*, 15 (1991) 553–559.

[18] Stohler, R., Rommelspacher, H., Ladewig, D. and Dammann, G., Beta-carboline (harman/norharman) sind bei heroinabhängigen erhöht, *Ther. Umschau*, 50 (1993) 178–181.

[19] Tanganelli, S., Antonelli, T., Morari, M., Bianchi, C. and Beani, L., Glutamate antagonists prevent morphine withdrawal in mice and guinea pigs, *Neurosci. Lett.*, 122 (1991) 270–272.

[20] Willcockson, W.S., Kim, J., Shin, H.K., Chung, J.M. and Willis, W.D., Actions of opioids on primate spinothalamic tract neurons, *J. Neurosci.*, 6 (1986) 2509–2520.