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European Journal of Pharmacology 509 (2005) 135–143

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Sensitization to morphine withdrawal in guinea-pigs

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Received 7 December 2004; accepted 24 December 2004
Available online 1 February 2005

Abstract

The aim of this study was to determine whether sensitization occurred to morphine withdrawal. Guinea-pigs were treated twice daily with increasing doses of morphine (10–100 mg/kg s.c.) for 3 days followed by injection of morphine 100 mg/kg on the fourth day. Sixty min after the last morphine injection, animals were withdrawn from morphine with naltrexone, 15 mg/kg s.c., and locomotor activity and all other behaviours scored over 90 min. Animals were then rested for 3 days. This procedure was repeated twice over the next 2 weeks. Control animals were treated with saline for the first two treatment cycles. Guinea-pigs subjected to three cycles of morphine withdrawal showed a significant increase in the total number of withdrawal behaviour counts over the 90-min observation period following the third cycle of withdrawal compared with the first and second withdrawal cycles. However, locomotor activity, a major sign of morphine withdrawal in guinea-pigs, was not significantly increased. Fos-LI was markedly increased in the repeatedly withdrawn animals in several brain regions, including amygdala, dorsal striatum, thalamus, ventral tegmental area, and ventrolateral periaqueductal gray area. It is concluded that sensitization to morphine withdrawal occurs in guinea-pigs.

Keywords: Morphine withdrawal; Opioid; Naltrexone; Sensitization; Fos; Guinea-pig

1. Introduction

It is now well established that repeated administration of morphine to rats induces sensitization to its motor stimulant effects (e.g., Kalivas and Duffy, 1987). It is also established that sensitization to opioids directly affects rewarding pathways in the brain, which play a role in drug seeking behaviour (Koob and Le Moal, 1997). However, despite extensive investigation of the morphine withdrawal response, no studies appear to have investigated whether sensitization occurs to morphine withdrawal, although it has recently been shown that sensitization occurs to ethanol withdrawal in mice (Veatch and Becker, 2002). Investigation of the sensitizing effect of repeated withdrawal from drugs of abuse is important since many human addicts undergo repeated episodes of withdrawal between drug administrations.

Morphine withdrawal produces behavioural activation and wide-spread expression of the inducible transcription factor, *c-fos*, and Fos-related proteins in the brain of both rats (Hayward et al., 1990; Erdtmann-Vourliotis et al., 1998; Georges et al., 2000) and guinea-pigs (Chahl et al., 1996). In the present study the behaviours of animals and the distribution of Fos-like immunoreactivity (Fos-LI) in the central nervous system following a single episode of morphine withdrawal were compared with those following three cycles of withdrawal. Guinea-pigs were used in these experiments, because like humans and unlike rats, they do not exhibit a motor stimulant response to morphine (Bot et al., 1992). They also have a distribution of brain opioid receptors that is more similar to human than rat (Mansour et al., 1988), and exhibit a marked and readily quantifiable antagonist-induced withdrawal from morphine (Chahl et al., 1996).

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doi:10.1016/j.ejphar.2004.12.043

2. Materials and methods

2.1. Treatment of animals

Ethics approval for the following experiments was obtained from the Animal Care and Ethics Committee of the University of Newcastle, an approved research establishment.

Eight male guinea-pigs, weighing between 400 and 750 g at the beginning of the experiment, were housed in individual cages, maintained at room temperature on a 12/12 h light/dark cycle, and allowed free access to food and water containing a vitamin C supplement. Animals were weighed daily throughout the experiment. The guinea-pigs were divided randomly into two groups: those that were subjected to one cycle of morphine withdrawal and those that were subjected to three cycles of morphine withdrawal. Guinea-pigs subjected to three cycles of morphine withdrawal were given one s.c. injection of morphine twice daily in the morning and afternoon during 3 days and one injection on the fourth day. To induce dependence and maintain effectiveness the doses of morphine were increased over the 3 days. For each cycle of morphine treatment the following doses of morphine were given: 10 mg/kg and 20 mg/kg on day 1, 40 mg/kg and 60 mg/kg on day 2, 80 mg/kg and 100 mg/kg on day 3, and 100 mg/kg on day 4. One hour after the last morphine injection on day 4, animals were given 15 mg/kg of the opioid antagonist, naltrexone hydrochloride s.c., to induce morphine withdrawal. This high dose of naltrexone was chosen because earlier unpublished experiments (Chahl, unpublished) showed that this dose produced maximum withdrawal in guinea-pigs following the high doses of morphine used in these experiments. It was considered important to obtain maximum withdrawal following the morphine treatment so that changing sensitivity to naltrexone itself would be less likely to be a variable. Following naltrexone injection the behaviour of the animals was observed for 90 min. The animals were then rested for 3 days, after which the above procedure was repeated twice (total of three cycles). Guinea-pigs subjected to one cycle of withdrawal were given equivalent volumes of saline instead of morphine for the first two cycles followed by naltrexone hydrochloride, 15 mg/kg, at the end of each cycle of saline treatment. During the third cycle these animals were given morphine instead of saline and withdrawn with naltrexone following the same procedure as for the animals subjected to three cycles of withdrawal.

Following the last 90-min observation period the animals were given an intraperitoneal injection of a lethal dose of sodium pentobarbitone (100 mg/kg). Ninety minutes was chosen as this is the time of peak Fos expression following neuronal activation (Morgan and Curran, 1991). The animals were then perfuse-fixed with 0.1 M phosphate-buffered saline (PBS, pH 7.4, 37 °C) containing 5000U/l heparin, followed by 4% paraformaldehyde in 0.1 M phosphate buffer. The brains were removed, post-fixed for 24 h at 4 °C and placed in 30% sucrose solution in 0.5% paraformaldehyde at 4 °C for cryoprotection until the brains sank.

2.2. Measurement of behaviour

Locomotor and other behavioural activities of guinea-pigs during withdrawal were measured in a modified animal cage equipped with a single infrared photocell and detector on the longitudinal axis (Bot et al., 1992). A digital counter recorded every crossing of the infrared beam at least 1.5 s apart and a single pulse record was made simultaneously on a chart recorder. Activity scores were obtained from the total number of counts over successive 10-min intervals throughout the experiment. The frequency of other behaviours characteristic of morphine withdrawal in guinea-pigs such as digging, face washing, rearing, head/body shake, scratching, vocalizing, sneezing, chewing, and stretching were recorded by a trained observer for each 10-min interval throughout the 90-min period following naltrexone injection.

2.3. Immunohistochemistry

Coronal sections, 50 μm, were cut on a cryostat at −17 °C and washed in PBS. Free floating sections were incubated in PBS containing 0.3% hydrogen peroxide for 20 min, washed in PBS, and incubated in 10% normal donkey serum (NDS) in PBS, containing 0.1% sodium azide, and 0.075% Triton X-100 for 30 min at room temperature. The sections were then incubated with rabbit Fos antiserum (c-Fos (K-25), Santa Cruz Biotechnology), diluted 1:2000 in 1% NDS in PBS containing 0.075% Triton X-100 and 0.1% sodium azide, for 48 h at 4 °C. The sections were washed with PBS three times for 15 min each, and then incubated with biotinylated donkey anti-rabbit IgG (Jackson ImmunoResearch Laboratories) diluted 1:1000 in 0.1% NDS in PBS containing 0.075% Triton X-100 and 0.1% sodium azide, for 1 h at room temperature. The sections were then washed with PBS three times, incubated with avidin–biotin–peroxidase complex (ABC, Vectastain Elite ABC kit, Vector Laboratories) in PBS for 1 h at room temperature, and washed three times with 0.05 M Tris-buffered saline (TBS, pH 7.4). To visualize bound antibodies, trays were cooled on ice and cold 0.05% 3,3′-diaminobenzidine (DAB, Sigma) in TBS containing 0.033% hydrogen peroxide, 0.004% nickel ammonium sulphate and 0.004% cobalt chloride was added to each well. After a brown colour had developed (2–4 min), the reaction was stopped by removing the DAB solution and adding deionized water to the wells. Sections were washed three times in deionized water, mounted on gelatin chrom alum-coated slides, air dried, dehydrated in ascending concentrations of ethanol (70%, 95%, 100%), cleared with histolene (Fronine, Riverstone, NSW, Australia) and cover-