Influence of 5-HT_3 receptor antagonists and the indirect 5-HT agonist, dexfenfluramine, on heroin self-administration in rats

Guy A. Higgins, Yehfat Wang, William A. Corrigall, Edward M. Sellers

Preclinical Pharmacology and Experimental Psychology Program, Addiction Research Foundation, 33 Russell Street, Toronto and Departments of Pharmacology, Physiology and Medicine, University of Toronto, Toronto, Ontario, Canada

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Abstract. The purpose of the present study was to examine the effects of the 5-HT_3 antagonists ondansetron and MDL72222, and the 5-HT releaser and reuptake inhibitor dexfenfluramine, on intravenous heroin self-administration by Wistar rats. Using separate squads of animals, two separate schedules of heroin reinforcement were used; a relatively low dose (0.03 mg/kg per infusion) made available under a FR5 schedule for 1 h each day, and a moderate heroin dose (0.1 mg/kg per infusion) available under a FR1 schedule for 2 h each day. Following the acquisition of stable levels of responding across days, both naloxone pretreatment (0.25 mg/kg SC) and halving the heroin infusion dose produced increases in operant responding for heroin at each concentration. Neither ondansetron (0.01-1 mg/kg SC) nor MDL72222 (0.1-3 mg/kg SC) pretreatment influenced heroin self-administration. Chronic treatment (5 day) of ondansetron (0.01-0.1 mg/kg) was similarly ineffective. However, dexfenfluramine (0.5-2.5 mg/kg IP) consistently reduced heroin self-administration at doses producing only modest decreases in food responding. These findings are in contrast to place conditioning studies, which show that 5-HT_3 antagonists but not indirect 5-HT agonists block a morphine-induced place preference. Reasons for such discrepancies remain to be determined.

Key words: Heroin self-administration – Rat – 5-HT – 2-HT_3 receptor antagonists – Ondansetron – MDL72222 – Indirect 5-HT agonist – Dexfenfluramine – Opioid reinforcement

Over recent years a considerable amount of progress has been made in understanding the neurobiology of opioid abuse behaviour (Wise and Bozarth 1987; Koob and Bloom 1988). Animal models which have been of particular value for this research include the conditioned place preference and intravenous opioid (principally heroin) self-administration paradigms (Carr et al. 1989; Koob and Goeders 1989). By the use of specific neurotoxin lesions, discrete central microinjections and selective pharmacological agents allied to these paradigms, a contributory role of the dopamine mesolimbic system in the maintenance of opioid reinforced behaviour has been established (Wise and Bozarth 1987; Carr et al. 1989; Koob and Goeders 1989), although an opioid component independent of dopaminergic influence has also been implicated, particularly in the maintenance of heroin self-administration behaviour (Ettenberg et al. 1982; Petit et al. 1984; Vaccarino et al. 1985). The involvement of other neuronal candidates, e.g. glutaminergic, GABAergic, which impinge on these systems has also been investigated (Koob and Goeders 1989; Hubner and Koob 1990; Pulviretti et al. 1992).

One relatively neglected research area has been to assess the effect of drugs which specifically interact with serotonergic (5-HT) systems as a means to modify opioid self-administration. This is despite reports that selective lesioning of 5-HT neurones within the nucleus accumbens, a terminal region within the mesolimbic system, prevents the development of morphine place conditioning (Spyraki et al. 1988) and increases morphine self-administration (Smith et al. 1987). 5-HT receptors are currently classified into at least four main subclasses (5-HT_1-4; see Peroutka 1993 for recent review). Perhaps the most likely pharmacological agents to influence opioid self-administration are selective antagonists at the 5-HT_3 receptor, e.g. ondansetron, MDL72222, and indirect 5-HT agonists, e.g. fluoxetine, dexfenfluramine. Various 5-HT_3 antagonists have been shown to block the acquisition of an opioid (morphine) conditioned place preference (Carboni et al. 1989; Higgins et al. 1992a). 5-HT agonists, notably the reuptake blocker fluoxetine, have been reported to reduce the self-administration of a diverse range of drug reinforcers such as alcohol, amphetamine and cocaine (Lecesse and Lyness 1984; Carroll et al. 1990; Richardson and Roberts 1991; Higgins et al. 1992b; Rowland and Morian 1992). To our knowledge, the effects of these agents against heroin self-administration has not previously been reported.

Correspondence to: G. A. Higgins, Glaxo Unit of Behavioural Psychopharmacology, University of Hertfordshire, College Lane, Hatfield, Herts, AL10 9AB, UK
Therefore, in the present study we have investigated the effect of the 5-HT3 antagonists ondansetron and MDL72222, and the 5-HT releaser/reuptake blocker dexfenfluramine, upon heroin self-administration. Dexfenfluramine was selected because in addition to blocking reuptake, this agent also directly releases 5-HT from nerve terminals and hence produces a more profound enhancement of 5-HT function by comparison with selective inhibitors of reuptake, e.g. fluoxetine (Fuller et al. 1988). Furthermore, unlike fluoxetine, the effects of dexfenfluramine are consistently shown to be blocked by 5-HT receptor antagonists (Rowland and Charlton 1985; Wong et al. 1988; Grignaschi and Samanin 1992; Higgins et al. 1992b). In a preliminary study (Higgins et al. 1993b) we reported that at a single dose of 1 mg/kg, dexfenfluramine produced a reduction in heroin self-administration. In the present studies we attempted to extend these observations: a low heroin dose of 0.03 mg/kg per infusion made available under a FR5 schedule for 1 h/day, and a moderate heroin dose of 0.1 mg/kg per infusion made available under a FR1 schedule for 2 h/day. The 5-HT3 receptor antagonists ondansetron and MDL72222 were tested in identical paradigms. Also, the effect of each of these treatments against food maintained responding was evaluated. A preliminary account of our findings with the 5-HT3 receptor antagonists has been presented to the British Pharmacological Society (Higgins et al. 1993a).

Materials and methods

Animals and housing. Male Wistar rats (Charles River, Quebec, Canada), starting weight 300 g, were used throughout. Upon arrival the animals were singly housed in hanging wire mesh cages within a holding room maintained at 22 ± 1°C and 50% humidity. The light period of the day-night cycle was 0700–1900 hours. Food (Lab diet, Richmond, Indiana) and water were available ad libitum for a habituation period of 5–7 days, after which the animals were food deprived overnight and trained to lever press for food reinforcement (45 mg Noyes pellets). Once trained, the animals were maintained for the duration of the study on approximately 20 g food per day, available as a single meal at 1700 hours. Under this regimen of food availability, the animals gained weight at the rate of approximately 15–20 g per month.

Apparatus. The self-administration chambers were constructed of stainless steel and perspex, and measured 22 × 22 × 28 cm (L × W × H) (Med Associates Inc.; VT, USA). Each chamber was equipped with two response levers mounted 7 cm above the gridded floor and positioned either side of a pellet dispenser which was only used during food shaping. Stimulus lights were located 5 cm above each lever. A houselight, positioned at the rear of the chamber provided illumination during test sessions. Reinforcement delivery was controlled by a microcomputer interface linked to a 386DX computer. Heroin infusions were made via the operation of a syringe pump (Razel) connected to PE60 tubing which passed through a counterbalanced swivel unit. From the swivel, the tubing was encaused within a heavy duty spring which screwed onto a pedestal assembly mounted on the animals back around the catheter (see Corrigall 1992 for further detail). This system allowed the rapid connection of animals to the drug delivery line.

Implantation of intravenous catheters. Following food shaping the FR5 schedule, the animals were surgically prepared with a chronic intravenous catheter (Corrigall 1992) under general anaesthesia with acepromazine maleate (Ayerst, 10 mg/kg IP) and ketamine HCl (Rogar STP, 100 mg/kg IM). The catheter was implanted into the jugular vein and exteriorised at the animals’ back, between the scapulae. All animals were allowed 7 days recovery before drug self-administration sessions commenced. Daily 0.1-ml infusions of heparinised saline (30 units/ml), usually made after each self-administration session, served to maintain catheter patency.

Heroin self-administration. The animals were first habituated to being connected to the spring and swivel assembly using food as the reinforcer. Following 1 day of such training, heroin self-administration training began. Only one lever was designated active during these sessions, although responses on both levers were recorded. Two separate schedules of reinforcement were run in separate squads of rats: i) a 0.03 mg/kg per infusion dose available for a 1-h period, and ii) a 0.1 mg/kg per infusion dose available under FR1 schedule for a 2-h period. At the 0.03 mg/kg per infusion dose, heroin was initially made available under an FR1 schedule. This value was increased over the course of training by increments of 1 to a final value of FR5. During self-administration sessions, heroin infusion was signalled by a stimulus light positioned above the designated active lever. Each infusion was followed by a 1-min time-out (TO) period, during which the houselight was switched off and lever presses were recorded but not reinforced. Heroin self-administration sessions were run daily, 7 days a week, and each animal was run at approximately the same time of the day. A single priming infusion was delivered by the experimenter at the start of each session.

Once stable rates of daily heroin self-administration had been attained, i.e. when the number of infusions did not vary by more than 3, for 3 consecutive days, drug testing began. At the 0.03 mg/kg per infusion dose this generally took approximately 25–30 days. Whereas for the 0.1 mg/kg per infusion dose, 15–20 sessions were required. With the exception of the chronic dosing studies, all drug testing was conducted according to a within-subjects design, with successive treatments separated by 1–3 days of baseline treatment. Drug treatments were given according to a pseudo-randomised design.

Operant responding for food reinforcement. Separate squads of animals, maintained under identical housing and food access conditions to those used in the heroin self-administration studies, were trained to respond for food reinforcement under an FR5TO1 min schedule for a 60-min period each day. Following the acquisition of stable rates of food responding (10–14 days), drug testing began according to a within-subjects design. Successive treatments were separated by 1–3 days of baseline (saline only).

Drugs and injections. Heroin hydrochloride (Ward Robertson, Scarborough, Ontario), Naloxone hydrochloride and MDL72222 (RBI, Natick, Mass.), ondansetron hydrochloride (Glaxo, Ware, UK), and dexfenfluramine hydrochloride (Servier) were each dissolved in 0.9% sodium chloride solution, except MDL72222 which was first mixed with a few drops of 0.1 N HCl before being made up to final volume with 0.9% sodium chloride. The final pH was then adjusted to 6 with 1 N NaOH. Final drug concentrations are expressed as that of the base, except dexfenfluramine, where the final concentration refers to that of the salt. Naloxone, ondansetron and MDL72222 were administered subcutaneously, dexfenfluramine was given intraperitoneally. A pretreatment time of 30 min was used for each test drug, except naloxone, which was administered 15 min before testing.

Analysis of data. In each study, the data are presented as the mean ± SEM for the group. The total number of infusions (20-min and 60-min readings at the low heroin dose, 2 h at moderate heroin dose) and the number of lever presses on both the active and inactive levers were measured. Statistical comparisons within groups in the low heroin dose study was made using two-way repeated measures ANOVA with drug dose and infusion time/or lever type as factors. Analysis of drug effects in the 0.1 mg/kg per infusion group was made by one-way repeated measures ANOVA with dose as factor.