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Buprenorphine is protective against the depressive effects of norbuprenorphine on ventilation

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Abstract

High dose buprenorphine is used as substitution treatment in heroin addiction. However, deaths have been reported in addicts using buprenorphine. The role of norbuprenorphine, an N-dealkyl metabolite of buprenorphine, was hypothesized to explain these fatal cases. We determined the median intravenous lethal dose (LD_{50}) of norbuprenorphine in male Sprague–Dawley rats. The effects of a single intravenous dose of 3 or 9 mg/kg norbuprenorphine alone on arterial blood gases were studied. Finally, the effect of pre- and post-administrations of buprenorphine on norbuprenorphine-induced changes on arterial blood gases were analyzed. Norbuprenorphine's LD_{50} was 10 mg kg^{-1}. Norbuprenorphine 3 mg kg^{-1} produces the rapid onset of sustained respiratory depression, as demonstrated at 20 min by a maximal significant increase in $PaCO_2$ (8.4 ± 0.9 versus 5.7 ± 0.1 kPa), decrease in arterial pH (7.25 ± 0.06 versus 7.44 ± 0.01), and hypoxia (8.3 ± 0.6 versus 11.1 ± 0.2 kPa). Buprenorphine not only protected against the effects of 3 mg kg^{-1} norbuprenorphine in a dose-dependent manner but also reversed the effects when given afterward. Binding experiments suggest a role for mu- and to a lesser extent for delta-opioid receptors in buprenorphine protective effect against norbuprenorphine-induced respiratory depression. In conclusion, our data clearly show that norbuprenorphine alone causes important deleterious effects on ventilation in rats. However, buprenorphine protective effect calls into question the role for norbuprenorphine in respiratory toxicity associated with buprenorphine use.

Keywords: Buprenorphine; Norbuprenorphine; Respiratory depression; Arterial blood gases; Median lethal dose; Cytochrome P450

Introduction

Heroin addiction is still responsible for large numbers of deaths (Henry, 1999). Substitution therapies, including methadone, levomethadyl acetate, and buprenorphine, provide successful maintenance therapy, with a substantial reduction in consumption of illicit opiate and psychoactive substances (Johnson et al., 2000). Since 1996, high dose (8–16 mg/day) of buprenorphine has been available in France as a substitution product for heroin addiction (Obadia et al., 2001; Gueye et al., 2002a). More recently, the US Food and Drug Administration approved buprenorphine as a maintenance therapy, suggesting a future large expansion of prescriptions on the American continent (Sporer, 2004). In spite of an estimated 90,000 patients treated actively with buprenorphine in France, the number of significant reported side effects has been limited. However, fatal cases have been reported in relation to buprenorphine "overdoses." Forensic studies concluded that these deaths were due to asphyxia, with the underlying cause attributed to misuse and/or co-administration of psychotropic substances (Tracqui et al.,

Abbreviations: BUP, buprenorphine; CYP, cytochrome P450; GC-MS, gas chromatographic-mass spectrometry; LD_{50}, median lethal dose; N-BUP, norbuprenorphine.

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1998; Gaulier et al., 2000; Kintz, 2001; Kintz, 2002; Pirnay et al., 2004). It is well known that buprenorphine is injected by some users, in spite of the labeling for sublingual use (Obadia et al., 2001; Comer et al., 2001; Kintz, 2002).

The exact mechanism of buprenorphine respiratory toxicity is still misunderstood. Experimental and clinical studies clearly demonstrate a dose–effect relationship for respiratory depression with a definite plateau (Cowan et al., 1977; Walsh et al., 1994; Ohtani et al., 1997). In a recent study, we showed that a single intravenous high dose of buprenorphine (up to 90 mg/kg), administered alone to naive rats, did not result in significant respiratory depression, whereas co-administration of a non-respiratory-depressant dose of midazolam (160 mg/kg, i.p.) significantly altered arterial blood gases (Gueye et al., 2002b).

Norbuprenorphine is an active metabolite derived from the N-dealkylation of buprenorphine, catalyzed in humans by cytochrome P450 (CYP) 3A4, mainly in the liver (Iribarne et al., 1997; Kobayashi et al., 1998). Human pharmacokinetic studies showed low plasma concentrations of norbuprenorphine following buprenorphine administration, but with peaks and elimination half-lives which varied markedly according to the routes of administration and among individuals (Kuhlman et al., 1996). In several fatal cases related to buprenorphine overdoses, high plasma or tissue concentrations of norbuprenorphine were reported, suggesting its role in the onset of death (Tracqui et al., 1998; Gaulier et al., 2000; Kintz, 2001, 2002; Pirnay et al., 2004). It is unclear whether norbuprenorphine alone can fully explain buprenorphine-related deaths. Indeed, buprenorphine CYP-mediated transformation into norbuprenorphine may be influenced in drug users by interactions with co-ingested psychoactive substances or otherwise altered by the modalities of abuse. Drugs that induce CYP3A, such as phenobarbital, carbamazepine, and phenytoin, could increase norbuprenorphine levels (Sporer, 2004). However, the clinical effects of these interactions are unknown. Recent experimental data in rats have shown that significant quantities of norbuprenorphine can be detected in the plasma, immediately after buprenorphine administration (Gopal et al., 2002), in contradiction to previous studies (Ohtani et al., 1994, 1995). Furthermore, significant respiratory depression has been demonstrated following the administration of a single intravenous dose of 3 mg kg^{-1} norbuprenorphine in rats (Ohtani et al., 1997).

To date, the acute toxicity and respiratory effects of norbuprenorphine are poorly understood. To address this issue, we undertook a series of studies, looking at the median lethal dose (LD_{50}) of intravenous norbuprenorphine and the effects of high dose of norbuprenorphine alone or in combination with buprenorphine on arterial blood gases in adult rats.

Materials and methods

All experiments were carried out within the ethical guidelines established by the National Institutes of Health and the French Minister of Agriculture.

Animals and drugs

Sprague–Dawley male rats (Iffa-Credo, France) weighing between 250 and 300 g at the time of experimentation were employed. They were housed during the 8 days before experimentation in a temperature- and light-controlled animal care unit. They were allowed food and water ad libitum until 1 day prior to experimentation. Buprenorphine and norbuprenorphine were generously supplied by Schering-Plough, SA. Buprenorphine was prepared in a concentration of 18.5 mg ml^{-1} in a mixture of 80% sterile water and 20% Tween 80. Norbuprenorphine was prepared in a similar manner at a concentration of 3.7 mg ml^{-1}. Ketamine (Ketalar, Parke Davis) and xylazine (Rompum, Bayer) were obtained from a veterinary supplier. The radioligands [^{3}H]-DAMGO (Tyr-D-Ala-Gly-(Me)Phe-Gly-ol) (51 Ci/mmol) and [^{3}H]-naltrindole (20 Ci/mmol) were purchased from Perkin-Elmer Life and Analytical Sciences (Courtaboeuf, France) and [^{3}H]-CI-977 (45 Ci/mmol) from Amersham Biosciences (Saclay, France).

Estimation of the mean lethal dose (LD_{50}) (Study 1)

To estimate the maximum non-lethal dose of norbuprenorphine, we determined the LD_{50} using an up-and-down method (Bruce, 1985). This method progressively brackets a target dose, in this case, the highest dose not resulting in mortality. Animals are treated one by one, and, according to outcome (death or survival), the dose for the subsequent animal is adjusted (decreased or increased, respectively). From the doses that do not result in death, one can then estimate the highest consistently non-lethal dose. The LD_{50} was determined on the basis of final dose, outcome/dose pattern, and dose interval. We determined these values in triplicate.

Approximately 18 h prior to experimentation, rats were fasted but allowed free access to water. At the time of experimentation, they were placed individually in horizontal Plexiglas cylinders (internal diameter: 65 cm, adjustable length up to 20 cm) (Harvard Apparatus, Inc. Holliston, MA, USA). The cylinders were modified with several additional openings on the cranial extremity to prevent CO_2 rebreathing. Norbuprenorphine was administered in non-anesthetized restrained animals via the tail vein. Following drug administration, they were placed in individual cages, allowed to eat and drink, and maintained in the laboratory, which was temperature-controlled with day lighting. Animals were examined repeatedly during the first 4 h after injection, then daily during a 7-day period, for mortality or evidence of drug-related side effects or other illness. At the end of the study period, they were euthanized using a carbon dioxide chamber.

Arterial blood gas studies (Studies 2 to 4)

Studies 2, 3, and 4 involved the determination of effects of norbuprenorphine on arterial blood gas values, either alone (Study 2), preceded or followed by a single dose of buprenorphine (Study 3), or preceded by various doses of buprenorphine (Study 4). The general study protocol was as follows: the day before the drug study, animals were anesthetized with 70 mg kg^{-1} ketamine and 10 mg kg^{-1} xylazine intraperitoneally then placed on a warming blanket with a regulating thermostat. A rectal probe permitted feedback control of temperature. The femoral vein and artery were catheterized using silastic tubing with external and internal diameters of 0.94 and 0.51 mm respectively and length of 30 cm (Dow Corning Co, MI, USA). The technique of catheterization has been previously described (Gueye et al., 2001). Rats were maintained during 24 h in their individual cages in order to completely recover after anesthesia. The day of experimentation, rats were placed individually in the horizontal Plexiglas cylinders. The venous catheter allowed administration of the study drug and the arterial catheter collection of arterial blood gases. Norbuprenorphine or aqueous solvent was administered in a volume of 1.2 ml by the femoral vein over 3 min by an infusion pump at a constant rate (Harvard Instruments-PHD 2000, USA). Respiratory rate and rectal temperature were measured in each treatment group. At each sampling time, the respiratory rate was counted for 1 min, the count being based on the observation of abdominal