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Buprenorphine and Midazolam Act in Combination to Depress Respiration in Rats

Papa N. Gueye,*[,1] Stephen W. Borron,*[,†,‡] Patricia Risède,* Claire Monier,* Françoise Buneaux,§ Marcel Debray,¶ and Frédéric J. Baud*

**INSERM U26 et Université Paris VII, Hôpital Fernand Widal, 200, rue du Faubourg Saint-Denis, 75475 Paris Cedex 10, France; †Department of Emergency Medicine, George Washington University, Washington, DC 20037; ‡International Toxicology Consultants, LLC, 2000 L Street NW, Suite 200, Washington, DC 20036–4924; §Laboratoire de Biochimie-Toxicologie, Hôpital Fernand Widal, 75475 Paris, France; and ¶Laboratoire de Biomathématiques, Université Paris V, UFR Pharmacie, 75006 Paris, France*

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High dose buprenorphine is used as substitution treatment in human heroin addiction. Deaths have been reported in addicts using buprenorphine, frequently in association with benzodiazepines. In the current study, we observed the effects of buprenorphine and midazolam alone and in combination on arterial blood gases. Four groups of 10 male Sprague-Dawley rats received a parenteral injection of aqueous solvent, buprenorphine (30 mg/kg, iv), midazolam (160 mg/kg, ip), or buprenorphine (30 mg/kg, iv) plus midazolam (160 mg/kg, ip). Serial blood gases were obtained over 3 hours. There was a mild but significant effect of buprenorphine alone in comparison with the aqueous solvent on $PaCO_2$ at 60 min (6.24 vs. 5.65 kPa, $p < 0.01$). There was also a mild but significant effect of midazolam alone in comparison with aqueous solvent on arterial pH at 90 min (7.33 vs. 7.41, $p < 0.001$) and $PaCO_2$ at 60 min (6.52 vs. 5.65 kPa, $p < 0.01$). The combination of midazolam and buprenorphine produces a rapid, profound, and prolonged respiratory depression, as demonstrated by an increase in $PaCO_2$ at 7.65 ± 0.12 kPa at 20 min and a decrease in arterial pH at 7.25 ± 0.02 at 20min, with appearance of delayed hypoxia with a decrease in PaO_2 at 8.74 ± 0.20 kPa at 120 min. These data show that high doses of midazolam and buprenorphine alone have limited effects on arterial blood gases in rats while midazolam and buprenorphine appear to act in an additive or synergistic fashion to depress ventilation in rats.

Key Words: **buprenorphine; midazolam; acute toxicity; respiratory depression; safety; rats; LD_{50}; arterial blood gas; opioids; heroin substitution; drug abuse.**

Heroin addiction remains a major concern throughout the world, and the number of deaths among heroin addicts, though difficult to assess, appears to have risen in a number of countries over the past decade (Battista *et al.,* 1993; Darke and Zador, 1996; Donoghoe and Hall, 1998; Hall and Darke, 1998; Hammersley *et al.,* 1995; Janssen *et al.*, 1989; Risser and Schneider, 1994; Steentoft *et al.,* 1989, 1996).

[1] To whom correspondence should be addressed. Fax: (+33) 1-49-96-65-78. E-mail: p.gueye@wanadoo.fr.

The approach to treatment of heroin addiction has undergone a profound evolution with the development of substitution treatments such as methadone, levomethadyl acetate, and buprenorphine. Products of substitution have been introduced in a number of countries with the goal of reducing mortality and morbidity associated with intravenous drug abuse, improving the addict's chances of reintegration into society. High dose (8–16 mg/day) buprenorphine has been available in France since 1996 and a recent report suggests that high-dose buprenorphine, like high dose methadone and levomethadyl acetate, substantially reduces the use of illicit opioids (Johnson *et al.,* 2000).

Dose-effect relationships of buprenorphine both in animals and humans suggest a plateau of respiratory effects (Cowan *et al.,* 1977; Walsh *et al.,* 1994) or no effect at all (Ohtani *et al.,* 1997). The plateau effect of buprenorphine appears of utmost importance regarding its safety for use in substitution treatment (Cowan *et al.,* 1977; Walsh *et al.,* 1994). In a previous study, we assessed the LD_{50} of intravenous buprenorphine in adult rats and measured the arterial blood gases in rats acutely exposed to high doses of buprenorphine (Gueye *et al.*, 2001). We did not observe any significant effect of the 3, 30, or 90 mg/kg doses of buprenorphine distinct from the effect of the aqueous solvent on arterial blood gases. It should be noted that 90 mg/kg approaches the minimum lethal dose (120 mg/kg) found in our LD_{50} study (Gueye *et al.*, 2001). In contrast with methadone, which induces potent respiratory depressant effects in rats (McCormick *et al.,* 1984), our data are consistent with a limited effect on respiration of a single intravenous dose of up to 90 mg/kg buprenorphine alone, as assessed by arterial blood gases.

However, deaths have been reported during substitution with buprenorphine in humans (Brenet *et al.,* 1998; Reynaud *et al.,* 1998; Tracqui *et al.,* 1998). Deaths may result from either misuse or overdose with substitution treatment (Robinson *et al.,* 1993; Tracqui *et al.,* 1998). In legitimate substitution treatment, buprenorphine is prescribed sublingually. However, there is now considerable evidence that buprenorphine is mis-

used and administered by the intravenous route (Robinson *et al.,* 1993).

Furthermore, recent reports have emphasized the combination of substitution products (methadone or buprenorphine) with psychotropic drugs as a major factor in fatalities among heroin addicts. More especially, the combination of buprenorphine and benzodiazepines is widely used by heroin addicts, and this combination is considered as a risk factor of lethal overdose (Drummer *et al.,* 1993; Hammersley *et al.,* 1995; Reynaud *et al.,* 1998; Tracqui *et al.,* 1998). However, benzodiazepines are considered relatively safe drugs and deaths caused by benzodiazepines alone in the absence of other pathology are uncommon (Drummer *et al.,* 1993; Finkle *et al.,* 1979; Serfaty and Masterton, 1993). In rats, diazepam alone did not cause any significant changes in arterial pH or $PaCO_2$. In contrast, there is a real potential for severe respiratory depression, as assessed by arterial blood gas measurement, when the combination of methadone and diazepam is given acutely to drug-naive rats (McCormick *et al.,* 1984).

To address the acute toxicity of buprenorphine in combination with midazolam, we performed the following study. We first determined the maximum nonlethal dose of intravenous midazolam in adult rats. Then, we studied the effects of buprenorphine and midazolam alone and in combination on respiratory rate and 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

Animals employed were Sprague-Dawley male rats (Iffa-Credo, France) weighing between 250 and 300 g at the time of experimentation. They were housed for 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.

Drugs

Buprenorphine hydrochloride was generously supplied by Schering-Plough, SA. It was subsequently diluted in a mixture of sterile water, ethanol, and hydrochloric acid 0.1 N at a pH of 4.0, at a concentration of 18.5 mg/ml, the greatest concentration achievable without further lowering the pH. This mixture of water, ethanol, and hydrochloric acid is referred to throughout this paper as "aqueous solvent."

Midazolam was generously supplied by Hoffman-La Roche, Inc. It was subsequently diluted in a mixture of sterile water and hydrochloric acid 0.1 N at a pH of 4.0.

Study 1: Determination of the Maximum Nonlethal Dose of Midazolam

To determine a toxic but nonlethal dose of midazolam, we first determine the LD_{50} of intravenous midazolam. Approximately 18 h prior to experimentation, the animals were fasted but allowed free access to water. The rats were placed individually in horizontal, Plexiglas cylinders (internal diameter: 6.5 cm, adjustable length: up to 20 cm, Harvard Apparatus, Inc., Massachusetts, U.S.). Midazolam was administered in awake, restrained animals via the tail vein. Animals were examined repeatedly during the first 4 h after injection, then daily, for evidence of drug-related side effects or other illness. Following drug administration, animals were placed in individual cages and allowed to eat and drink, and were maintained in the laboratory, which was temperature-controlled with day lighting.

Animals were observed for a period of 7 days after midazolam injection. At the end of the study period, animals were euthanized using a CO_2 chamber.

The up-and-down method, as proposed by Dixon (Dixon 1991; Dixon and Mood 1948) and refined by Bruce (Bruce, 1985, 1987), was employed to determine the LD_{50} in triplicate, calculated on the basis of final dose, outcome/dose pattern, and dose interval.

Study 2: Effects of a High Dose of Buprenorphine or Midazolam Alone and in Combination on Respiratory Rate and Arterial Blood Gases

Catheterization. The day before the study, the animals were anesthetized with ketamine (Ketalar®), 70 mg/kg, and xylazine (Rompum®), 10 mg/kg, intraperitoneally, then placed on a warming blanket with a regulating thermostat. A rectal probe permitted feedback control of the temperature. The femoral vein and artery were catheterized with Silastic® tubing with external and internal diameters of 0.94 and 0.51 mm, respectively; length 30 cm (Dow Corning Co, Michigan). The technique of catheterization was described elsewhere (Gueye *et al.,* 2001).

The day of experimentation, rats were placed individually in horizontal Plexiglas cylinders (internal diameter: 6.5 cm, adjustable length up to 20 cm, Harvard Apparatus, Inc., Massachusetts). The Plexiglas cylinders are provided with several openings on the cranial end in order to prevent CO_2 rebreathing.

Drug administration and collection of arterial blood gases. The venous catheter permitted the administration of the study drug. The arterial catheter permitted blood collection for arterial blood gases. Midazolam (160 mg/kg) was administered by the intraperitoneal route. Buprenorphine (30 mg/kg) or aqueous solvent, in a volume of 1.2 ml, was administered by the femoral vein over 3 min by an infusion pump at a constant rate (Harvard Instruments–PHD 2000, USA). In combination, midazolam was first administered, ip, followed 30 min later by the iv injection of buprenorphine. For the measurement of arterial blood gases, blood samples of 300 μl were collected in a heparinized syringe from the arterial catheter. Arterial blood samples were collected before and at 5, 20, 60, 90, 120, and 180 min after the administration of the drug, and immediately measured by means of a blood gas analyzer (Radiometer ABL 300, Copenhagen, Denmark). The first blood sample collected after injection in the midazolam group was 35 min after intraperitoneal injection to allow for direct comparison between groups. This delay permitted the onset of coma in animals having received midazolam.

Respiratory rate. At each sampling time, the respiratory rate was counted for 1 min, the count being based on the up-and-down movement of the abdomen caused by the animal's breathing.

At the end of experiments, rats were euthanized by the injection of a lethal dose of sodium pentobarbital.

Statistical Analysis

Four groups (aqueous solvent, midazolam, buprenorphine, and combination groups) of 10 restrained animals were used. The results are expressed as mean ± SEM. Baseline values were compared using one-way analysis of variance, followed by multiple comparison tests using Bonferroni's correction. In each group, comparisons of drug effects to baseline values were performed using repeated measures ANOVA and Dunnett's multiple comparison tests. The effects of the acute administration of buprenorphine or midazolam alone were compared to those of the aqueous solvent. Then, the effects of buprenorphine alone or midazolam alone were compared to those induced by the two in combination. Then, for each sampling time and each drug we calculated the difference between the value at that time and its corresponding baseline value. These differences were compared using one-way analysis of variance followed by multiple-comparison tests using Bonferroni's correction. All tests were performed using Prism version 2.0 (GraphPad Software, Inc., San Diego, CA), were 2-tailed, and p values of less than 0.05 were considered significant.

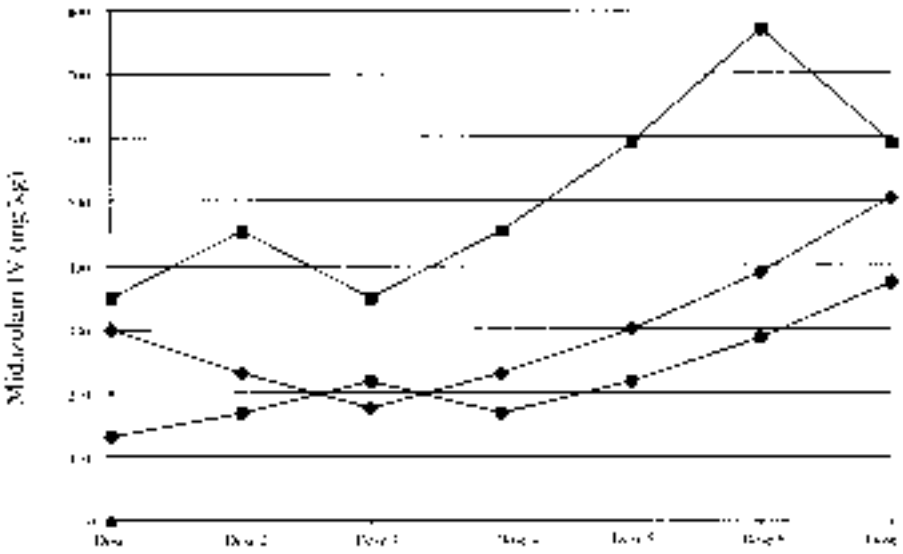

FIG. 1. Median lethal dose of intravenous midazolam in 3 series of opiate-naive adult rats. Each upward movement of the graph indicates survival of the previous animal, whereas a downward movement implies the death of the previous animal.

RESULTS

Study 1 Determination of the Maximum Nonlethal Dose of Midazolam

The LD_{50} obtained for the 3 series were 322, 357, and 555 mg/kg (Fig. 1). The median for the 3 groups was 357 mg/kg. Deaths occurred rapidly following injection (within 1–3 min) in all but one animal, which died within 24 h. Deep coma and apnea were the apparent cause of death. The mean dose received by surviving animals was 321 ± 38 mg/kg. The minimal dose that induced coma in this study was 130 mg/kg.

Study 2: Effects of Buprenorphine or Midazolam Alone and in Combination

Behavior. Control animals receiving aqueous solvent remained alert during the entire study period. Animals given 30 mg/kg of buprenorphine also remained alert during the entire study period. Rats that received 160 mg/kg, ip, of midazolam alone or combined with buprenorphine were in a deep coma at the 10- to 20-min period after injection, and were not awake until 180 min post-injection. One animal in the combination group died at 150 min post-injection.

Respiratory rate: Baseline values before treatment. There were no significant differences of the baseline values of the respiratory rate in the 4 treatment groups.

Comparison with baseline values. In the aqueous solvent and buprenorphine groups, there were no significant differences in the respiratory rate at any time in comparison with the baseline value. In the midazolam group, the respiratory rate was significantly lower than the baseline value at only 5 min post-injection (84 ± 7/min vs. 107 ± 2/min: $p < 0.01$). In the combination group, the respiratory rate was significantly lower than the baseline value at all times ($p < 0.01$); the lowest mean respiratory rate was recorded at 20 min post-injection (76 ± 5/min vs. 112 ± 5/min, respectively).

Effects of treatment at each sampling time. The respiratory rate in the combination group was significantly lower than that in the buprenorphine group at 20 min (76 ± 5/min vs. 101±3/min, respectively: $p < 0.05$) and 60 min (77 ± 4/min vs. 104 ± 4/min, respectively: $p < 0.01$). The respiratory rate in the combination group was significantly lower than that in the midazolam group at 120 min (86 ± 6/min vs. 104 ± 4/min, respectively: $p < 0.05$; Fig. 2).

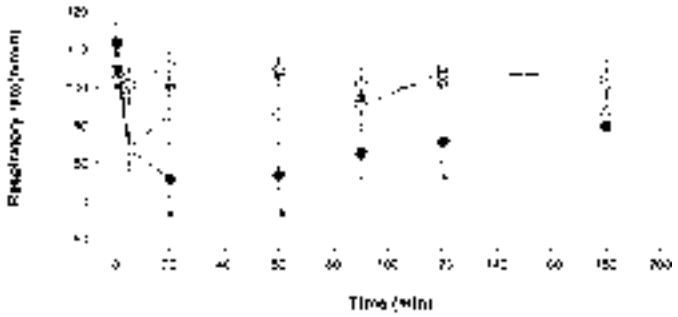

FIG. 2. Effects of aqueous solvent (open circle), midazolam 160 mg/kg, ip (open square), buprenorphine 30 mg/kg, iv (open triangle), and combination of midazolam and buprenorphine (filled circle) on the respiratory rate. In each group, 10 rats were used. Values represent mean ± SEM at each post-injection time interval. The comparison of the effects of treatment at each sampling time showed that the respiratory rate in the combination group was significantly lower than that in the buprenorphine group at 20 and 60 min ($p < 0.05$), and than that in the midazolam group at 120 min ($p < 0.05$). The differences between groups at each sampling time were compared using one-way analysis of variance followed by multiple comparison tests and Bonferroni's correction; *$p < 0.05$.

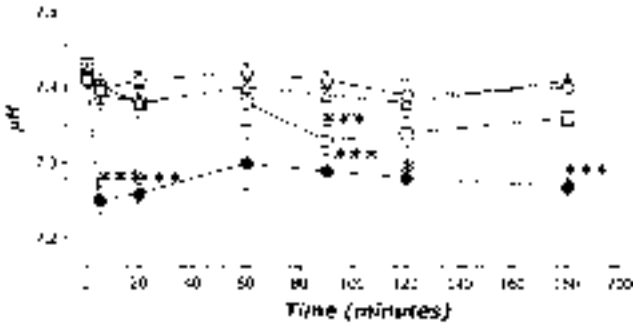

FIG. 3. Effects of aqueous solvent (open circle), midazolam 160 mg/kg, ip (open square), buprenorphine 30 mg/kg, iv (open triangle), and combination of midazolam and buprenorphine (filled circle) on the arterial pH. In each group, 10 rats were used. Values represent mean ± SEM at each post-injection time interval. The comparison of the effects of treatment at each sampling time showed that the arterial pH in the combination group was significantly lower than that in the buprenorphine group at 5, 20, 90 ($p < 0.001$), 120 ($p < 0.05$), and 180 min ($p < 0.001$), and than that in the midazolam group at 5, 20 ($p < 0.001$), and 180 min ($p < 0.05$). The differences between groups at each sampling time were compared using one-way analysis of variance followed by multiple comparison tests using Bonferroni's correction; *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$.

Arterial Blood Gases

Baseline values before treatment. There were no differences when comparing the baseline values of arterial pH, $PaCO_2$, PaO_2, or blood bicarbonate concentrations between the tested groups before treatment.

Comparison with Baseline Values

The aqueous solvent group. There were no significant effects in this treatment group on the arterial pH, $PaCO_2$, and blood bicarbonate concentrations in comparison with the baseline values. The PaO_2 value at 5 min was slightly but significantly lower than the baseline value (10.74 ± 0.54 kPa vs. 11.95 ± 0.15 kPa, $p < 0.05$).

The midazolam group (160 mg/kg, ip). When compared with baseline values, this group showed significant differences on the arterial pH from 90 min post-injection, on $PaCO_2$ at 60 and 90 min, on blood bicarbonate concentrations from 60 min, and on PaO_2 at 90 and 180 min.

The buprenorphine group (30 mg/kg, iv). In comparison with the baseline values, this group had significant differences in the arterial pH at 20 and 60 min, and in the PaO_2 at 5 and 20 min. There was no significant effect on the $PaCO_2$ or blood bicarbonate concentrations compared with the baseline values.

The combination group. In comparison with the baseline values, there were significant differences on the arterial pH and the $PaCO_2$ at all times in this group. The blood bicarbonate concentrations were significantly lower in comparison with the baseline value at 5, 120, and 180 min. The PaO_2 values were significantly lower in comparison with the baseline value at 5, 90, 120, and 180 min.

Effects of Treatment at Each Sampling Time

pH. There were no significant differences of arterial pH between the aqueous solvent and midazolam or buprenorphine groups, except at 90 min when the arterial pH in the midazolam group was significantly lower than that of the aqueous solvent ($p < 0.001$; Fig. 3). The lowest pH values in the aqueous solvent, midazolam, and buprenorphine groups were 7.39 ± 0.02 at 120 min, 7.33 ± 0.01 at 90 min, and 7.38 ± 0.01 at 120 min, respectively.

In comparison with the buprenorphine and midazolam groups, the arterial pH in the combination group significantly decreased at 5 min ($p < 0.001$) and remained decreased throughout the observation period (Fig. 3). The lowest pH value in the combination group was 7.25 ± 0.02 at 5 min.

$PaCO_2$. The $PaCO_2$ in both midazolam and buprenorphine groups was significantly greater than that in the aqueous solvent group at only 60 min ($p < 0.01$) (Fig. 4). The greatest $PaCO_2$ values in the aqueous solvent, midazolam, and buprenorphine groups were 6.10 ± 0.33 kPa at 5 min, 6.52 ± 0.17 kPa at 60 min, and 6.43 ± 0.31 kPa at 20 min, respectively.

In comparison with the buprenorphine and midazolam groups, the $PaCO_2$ in the combination group significantly increased at 5 min ($p < 0.01$) and remained significantly increased up to 60 min post-injection ($p < 0.001$; Fig. 4). The greatest $PaCO_2$ value in the combination group was 7.65 ± 0.12 kPa at 20 min.

Blood bicarbonate concentrations. The blood bicarbonate concentrations in the midazolam group were significantly lower than in the aqueous solvent group at only 180 min ($p < 0.05$). There were no significant differences of the blood bi-

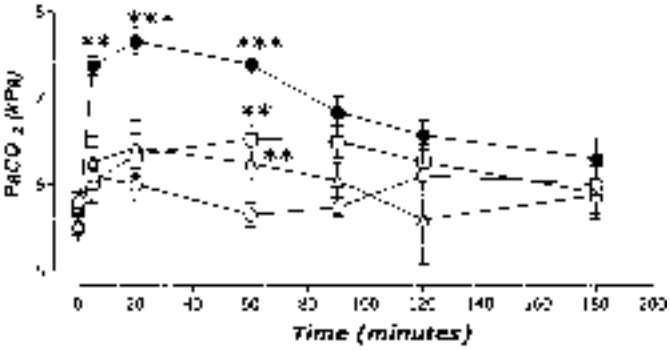

FIG. 4. Effects of aqueous solvent (open circle), midazolam 160 mg/kg, ip (open square), buprenorphine 30 mg/kg, iv (open triangle), and combination of midazolam and buprenorphine (filled circle) on the $PaCO_2$. In each group, 10 rats were used. Values represent mean ± SEM at each post-injection time interval. The comparison of the effects of treatment at each sampling time showed that the $PaCO_2$ in the combination group was significantly greater than those in both midazolam and buprenorphine groups at 5 ($p < 0.01$), 20, and 60 min ($p < 0.001$). The differences between groups at each sampling time were compared using one-way analysis of variance followed by multiple comparison tests using Bonferroni's correction; *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$.

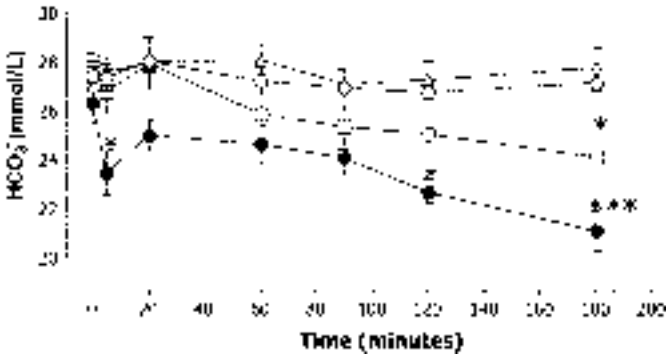

FIG. 5. Effects of aqueous solvent (open circle), midazolam 160 mg/kg, ip (open square), buprenorphine 30 mg/kg, iv (open triangle), and combination of midazolam and buprenorphine (filled circle) on the blood bicarbonate concentrations. In each group, 10 rats were used. Values represent mean ± SEM at each post-injection time interval. The comparison of the effects of treatment at each sampling time showed that the blood bicarbonate concentrations in the combination group were significantly lower than that in the buprenorphine group at 5, 120 ($p < 0.05$), and 180 min ($p < 0.001$). The differences between groups at each sampling time were compared using one-way analysis of variance followed by multiple comparison tests and Bonferroni's correction; $*p < 0.05$, $**p < 0.01$, $***p < 0.001$.

carbonate concentrations between the aqueous solvent and buprenorphine groups at any time (Fig. 5). The lowest blood bicarbonate concentration values in the aqueous solvent, midazolam, and buprenorphine groups were 26.8 ± 0.3 mmol/l at 120 min, 24.2 ± 0.9 mmol/l at 180 min, and 27.2 ± 0.6 mmol/l at 90 min, respectively.

There was a trend for blood bicarbonate concentrations in the combination group to decrease in comparison with the buprenorphine group. The decrease became statistically significant at 120-min post-injection ($p < 0.05$; Fig. 5). The lowest blood bicarbonate concentration in the combination group was 21.1 ± 0.8 mmol/l at 180 min.

PaO_2. There were no significant differences of the PaO_2 between the aqueous solvent and midazolam or buprenorphine group at any time (Fig. 6). The lowest PaO_2 values in the aqueous solvent, midazolam, and buprenorphine groups were 10.74 ± 0.54 kPa at 5 min, 11.40 ± 0.22 kPa at 20 min, and 10.37 ± 0.36 kPa at 20 min, respectively.

In comparison with the buprenorphine and midazolam groups, there was a progressive decline of the PaO_2 in the combination group that became statistically significant at 90-min post-injection ($p < 0.001$; Fig. 6). The lowest PaO_2 value in the combination group was 8.74 ± 0.20 kPa at 120 min.

DISCUSSION

Opioids, both natural and synthetic, have been shown to induce respiratory acidosis and hypoxia. Low doses of morphine or sufentanil induced respiratory depression in rats, as evidenced by a rapid increase in $PaCO_2$, without major effects on PaO_2 (Verborgh *et al.,* 1998). In doses ranging from 0.30 to 30 mg/kg, morphine linearly increased the $PaCO_2$ and reduced PaO_2 in rats (Cowan *et al.,* 1977). Similarly, acute administration of methadone (5 mg/kg, ip) caused a significant decrease in arterial pH and PaO_2, and an increase in $PaCO_2$ in rats (McCormick *et al.,* 1984). In contrast to these significant respiratory effects of morphine and methadone, buprenorphine has limited effects on respiration. Indeed, data collected in rats and mice following the administration of a single dose of buprenorphine, with regard to both the respiratory rate and the arterial blood gases, are consistent with either a plateau effect (Cowan *et al.,* 1977) or no effect at all (Ohtani *et al.,* 1997). In rats, buprenorphine (0.01–10 mg/kg intra-arterially) increased arterial $PaCO_2$ and reduced PaO_2 at 15 min (Cowan *et al.,* 1977). However, the dose-effect relationship showed that depression of respiration reached a plateau over the dose range 0.10–10 mg/kg (Cowan *et al.,* 1977). Ohtani *et al.* (1997) studied the respiratory effects of an iv bolus of buprenorphine in the range of 0.008 to 3 mg/kg in rats. Neither the respiratory rate nor the arterial $PaCO_2$ level changed over the dose range studied. Furthermore, during the continuous intravenous infusion of buprenorphine in rats (20 mg/kg/h), the respiratory rate gradually decreased, with a minimum respiratory rate being observed 3 h after the beginning of infusion. However, the decrease was not statistically significant, even at the highest infusion rate (Ohtani *et al.,* 1997). In a previous study we found a median lethal dose of intravenous buprenorphine of 146.5 mg/kg in adult Sprague-Dawley rats (Gueye *et al.,* 2001). In the present study, we used a 30-mg/kg dose of buprenorphine, a dose far greater than that used in the study by Ohtani *et al.* (1997). We observed a very mild and transient effect of a 30-mg/kg dose of buprenorphine on the $PaCO_2$ in

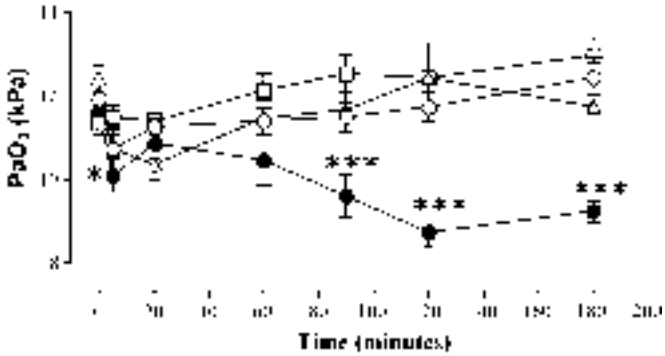

FIG. 6. Effects of aqueous solvent (open circle), midazolam 160 mg/kg, ip (open square), buprenorphine 30 mg/kg, iv (open triangle), and a combination of midazolam and buprenorphine (filled circle) on the PaO_2. In each group, 10 rats were used. Values represent mean ± SEM at each post-injection time interval. The comparison of the effects of treatment at each sampling time showed that the PaO_2 in the combination group was significantly lower than that in the midazolam group at 5 min ($p < 0.05$), 90, 120, and 180 min ($p < 0.001$), and than that in the buprenorphine group at 120 and 180 min ($p < 0.05$). The differences between groups at each sampling time were compared using one-way analysis of variance followed by multiple comparison tests using Bonferroni's correction; $*p < 0.05$, $**p < 0.01$, $***p < 0.001$.

comparison with the aqueous solvent. Our data and those from others are consistent with a limited effect of a single intravenous dose of buprenorphine on respiration, as assessed by arterial blood gases.

Benzodiazepines have been shown to be relatively free of severe respiratory effects when used alone (McCormick *et al*., 1984; Bahar *et al*., 1997; Verborgh *et al*., 1998). Midazolam is a benzodiazepine commonly used as an induction agent or to provide sedation. In an initial phase of our study, we assessed the acute toxicity of midazolam, finding an intravenous LD_{50} of 357 mg/kg, far greater than that previously reported (Schläppi 1983). Our LD_{50} study demonstrated a low toxicity of intravenous midazolam alone in rat. In the subsequent blood-gas study, we used a dose of midazolam (160 mg/kg, ip), which alone reproducibly induced a deep coma with zero mortality and moderate respiratory depression. Indeed, the highest $PaCO_2$ was 6.52 ± 0.17 kPa while the lowest arterial pH was 7.33 ± 0.01 after 90 min. An important finding was that in spite of respiratory acidosis, the PaO_2 did not change following midazolam administration while the animals were breathing room air. In fact, an increase in PaO_2 has been reported in rats following subcutaneous administration of 10 mg/kg chlordiazepoxide (Verborgh *et al*., 1998), or ip administration of 20 mg/kg diazepam (McCormick *et al*., 1984). A decrease in oxygen consumption may explain the lack of effect on PaO_2. Indeed, a decrease in oxygen consumption has been shown in man following the administration of anesthetic doses of various benzodiazepines (Marty *et al*., 1986; Nitenberg *et al*., 1983). The greatest decrease in oxygen consumption in man was observed with midazolam (Marty *et al*., 1986; Mohan *et al*., 1988).

The doses used in this study were far greater (on a mg/kg basis) than those used in humans. Indeed, the doses of buprenorphine and midazolam used in this animal study were in the toxic range. Rats received 160 mg/kg midazolam, ip, while sedative doses used in humans range between 0.05 to 0.2 mg/kg (Forster *et al*., 1983; Morel *et al*., 1984). Our rats received 30-mg/kg buprenorphine, iv. In adult humans, buprenorphine is used as an analgesic at the recommended parenteral dose of 0.3 to 0.6 mg (Watson *et al*., 1982). In substitution treatment of opioid addiction, the doses typically range between 2 and 16 mg sublingually, daily, which corresponds to approximately 0.03 to 0.23 mg/kg (Johnson *et al*., 1995). However, Walsh showed in healthy adult male volunteers a plateau of respiratory effect by sublingual buprenorphine at doses up to 32 mg (Walsh *et al*., 1994). Our study showed that large doses of buprenorphine and midazolam alone induced limited effects on respiratory rate and arterial blood gases in rats. However, the two in combination induced a severe respiratory depression as assessed by frank respiratory acidosis and hypoxemia. The lowest arterial pH, 7.25 ± 0.02, occurred within 5 min after the combination of midazolam + buprenorphine treatment, while the greatest $PaCO_2$, 7.65 ± 0.12 kPa, was recorded 20 min later. Both the arterial pH and the $PaCO_2$ improved over time but did not return to control values, even at the end of the study. Similarly, McCormick *et al* reported severe and long-lasting respiratory acidosis following the administration of 20-mg/kg diazepam subcutaneously in association with 5 mg/kg of methadone intraperitoneally. Furthermore, Verborgh *et al*. (1998) showed that the combination of chlordiazepoxide with either morphine or sufentanyl significantly increased the $PaCO_2$. However, in contrast to buprenorphine, both morphine and methadone are known to significantly depress respiration when administered alone (McCormick *et al*., 1984; Verborgh *et al*., 1998). Our results and those of others (Cowan *et al*., 1977; Ohtani *et al*., 1997) showed limited or no effect of buprenorphine alone on arterial blood gases in rats. Thus, we conclude that the limited and transient effect on respiration of high-dose buprenorphine alone does not hold when used in combination with midazolam. In fact, buprenorphine and midazolam appear to act in at least an additive fashion to depress respiration in rats.

Numerous reports have shown evidence of significant interactions between benzodiazepines and opioids with regard to analgesia (Dosaka-Akita *et al*., 1992; Paakkari *et al*., 1993). These interactions seem rather complex, as some reports indicated potentiation of the effects, while others suggested antagonism (Bradshaw *et al*., 1973; Brady *et al*., 1984; Shannon *et al*., 1976). It has long been recognized that the respiratory depression induced by opiates may be aggravated by the addition of sedative-hypnotic drugs such as benzodiazepines or barbiturates. In spite of the awareness of this phenomenon, little is known about the quantitative aspects of these relationships. Our data show that 2 drugs with limited and transient effects on respiration can induce a severe, prolonged respiratory depression when used in combination.

A number of mechanisms have been suggested to explain the interaction of opioids and benzodiazepines. An interaction may result from a pharmacokinetic or a pharmacodynamic process. The major metabolite of buprenorphine, norbuprenorphine, was shown to have a severe respiratory depressant effect (Ohtani *et al*., 1997). However, we are not aware of any study dealing with the metabolic interactions of buprenorphine or norbuprenorphine with midazolam. At pharmacological doses, midazolam is metabolized in the rat by cytochrome P450 3A1 and 3A2 (Greenblatt and Abernethy, 1985) while buprenorphine is metabolized by cytochrome P450 3A4 (Kobayashi *et al*., 1998; Kilicarslan, and Sellers, 2000). Furthermore, it seems unlikely that metabolic interactions may explain the rapid onset, 5 min after buprenorphine injection, of the respiratory depression observed in our study.

The opioids appear to depress respiration through their agonist activity at μ and δ receptors (Shook *et al*., 1990) while the κ receptor agonists appear to be protective (Dosaka-Akita *et al*., 1992; Shook *et al*., 1990). The benzodiazepines act at the $GABA_A$ receptors (Gravish and Snyder, 1980; Haefeley, 1990). The potential for effects of certain benzodiazepines at the opiate receptors and vice versa has been demonstrated

(Brady *et al*., 1984; Rodgers *et al*., 1985). Indeed, the antinociceptive effects of morphine may be diminished by the administration of flumazenil, a specific benzodiazepine receptor antagonist (Brady *et al*., 1984). Conversely, the diminished activity noted after administration of the benzodiazepine chlordiazepoxide, when administered in high doses (20 mg/kg) could be further diminished by the administration of the specific μ-antagonist naloxone (Rodgers *et al*., 1985). However, receptor specificity is typically studied at pharmacological doses. We cannot assume that these relationships hold at the high doses employed in our study.

Nonspecific factors may also explain our findings. Stress may antagonize or mask the respiratory effects of opiates (Hanks and Twycross, 1984). Several authors have suggested that benzodiazepines may reduce the stress, increasing the magnitude of opiate-induced respiratory depression (Van den Hoogen *et al*., 1989; Verborgh *et al*., 1998).

Finally, it is worth noting that delayed significant hypoxia was observed in the combination group, even after other respiratory parameters began to improve. In the absence of respiratory physiological studies, we cannot be sure of the mechanisms of this effect.

Conclusion

Buprenorphine and midazolam alone, in the doses tested, induce a mild and transient respiratory depression in comparison with the aqueous solvent. In contrast, the combination of midazolam and buprenorphine produces a rapid, profound, and prolonged respiratory depression as demonstrated by the early and sustained increase in $PaCO_2$, decrease in arterial pH, and delayed decrease in PaO_2. These data suggest that midazolam and buprenorphine act in an at least additive fashion to depress ventilation in rats.

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