Kinetics of Respiratory Depression in Rats Induced by Buprenorphine and its Metabolite, Norbuprenorphine

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ABSTRACT

The respiratory depression induced by buprenorphine and its active metabolite, norbuprenorphine (NBN), was evaluated in rats by measurement of changes in respiratory rate and arterial PCO₂ levels. After i.v. bolus administration of buprenorphine no effects were noted over the dose range 0.008 to 3 mg/kg; by contrast, the respiratory rate after rapid i.v. administration of NBN decreased in a dose-dependent fashion within the dose range of 1 to 3 mg/kg, and the arterial PCO₂ levels also varied in relation to the change in respiratory rate. The minimum respiratory rate was observed 15 min after NBN administration. Judging by the respiratory depressive effect after i.v. infusion, NBN was approximately 10 times more potent than the parent drug. In spite of the similarity of NBN concentrations in the brain after i.a. and after i.v. administration of NBN (3 mg/kg), neither the respiratory rate nor the arterial PCO₂ levels after i.a. administration changed compared with the control levels. Moreover, the NBN concentration in the lungs after i.v. administration was approximately 4-fold higher than that after i.a. administration. NBN-induced depression was rapidly reduced after i.v. administration of naloxone and β-funaltrexamine, but ICI 174864 was without effect. These results suggest that the respiratory depression induced by NBN may be mediated by opioid μ receptors in the lung rather than in the brain.

Materials and Methods

Chemicals. The hydrochloride salts of BN, NBN and N-propyl-norbuprenorphine (used as an analytical internal standard) were kindly supplied by Otsuka Pharmaceuticals Co. (Osaka, Japan). Naloxone hydrochloride was purchased from Sankyo Pharmaceuticals Co. (Tokyo, Japan). β-FNA was purchased from Peninsula Lab. Inc. (San Carlos, CA) and ICI 174864 from Cambridge Research Biochemicals (Cambridge, UK). PFPA was obtained from Pierce Chemical (Rockford, IL). All other solvents and reagents used were commercially available and of analytical grade.

Animals. Male Wistar rats (Japan Laboratory Animals Co., Tokyo) weighing 200 to 230 g were used. Animals were housed in well-ventilated cages at 20°C–22°C for at least 1 week before the experiments. Food (a solid feed MF, Orient Yeast Co., Tokyo) and water were available ad libitum.

Tissue distribution. Polyethylene cannulas (PE-50, Becton Dickinson, Parsippany, NJ) were inserted into the left femoral artery and vein under light anesthesia with diethyl ether. After surgery, the animals were left for 1.5 to 2 hr to recover. A dose of BN (0.6 mg/kg) or NBN (0.6 mg/kg) was then administered through the venous cannula over a 20-sec period. Subsequently, venous blood was collected...
collected (1 ml) in heparinized tubes through the arterial cannula at 2, 5, 15, 60, 120, 180 and 240 min after drug administration, and then the animal was sacrificed by decapitation, followed by immediate removal of the brain. Plasma (0.5 ml) was immediately separated from the blood by centrifugation at 1620 \times g for 5 min, and the plasma and tissue samples were stored at -80°C until analysis. The tissue samples were homogenized on ice with 3 volumes of ice-cold 1.15% KCl aqueous solution just before analysis. Twenty-two rats were used in each administration group.

Plasma concentration-time profiles. Intravenous Bolus Administration. Polyethylene cannulas were inserted into the left femoral artery and vein in the same manner as described above. After surgery, the animals were left for 1.5 to 2 hr. A dose of BN (0.08, 0.06, 0.6, 1, 2 or 3 mg/kg) or NBN (0.01, 0.6, 1, 2 or 3 mg/kg) was then administered through the venous cannula over a 20-sec period. Arterial blood samples (1 ml) were collected at 2, 5, 15, 30, 60, 120, 240, 300 and 480 min after drug administration. Individual rats contributed anywhere from 1 to 3 blood samples. Fifteen rats were used in each administration group.

Intra-arterial Bolus Administration. A polyethylene cannula was carefully inserted into the left ventricular artery through the left jugular vein. Then the cannulation into the left femoral vein was performed as described above. A dose of NBN (1, 2 or 3 mg/kg) and physiological saline (1 ml/kg) were simultaneously administered through the ventricular cannula and the femoral vein cannula over a 20-sec period. In order to compare the results with those of the i.a. administration experiments, i.v. administration experiments were performed using sham-operated rats. The same doses of NBN and physiological saline were administered through the femoral cannula and the ventricular cannula, respectively. The respiratory rate of the rats was measured before, and until 280 min after, drug administration. In another experiment, after the collection of venous blood 15 min after the administration of NBN (3 mg/kg dose), animals were sacrificed by decapitation, and the brain, heart and lungs were immediately removed. Plasma was separated from the blood in the manner described above, and the plasma and tissue samples were stored at -80°C until analysis. The tissue samples were homogenized in the same way as described above just before analysis.

Measurement of respiratory rate and arterial pCO2 after i.v. and i.a. bolus administration. Blood samples (0.3 ml) for measuring pCO2 by a blood gas analyzer (Radiometer Co., Tokyo) were collected through the femoral artery cannula before, and at 5, 30, 60, 120, 180, 240, 360 and 480 min after, the i.v. or i.a. administration of BN (0.06–3 mg/kg) and before, and at 5, 15, 30, 60, 120, 180 and 240 min after the i.v. or i.a. administration of NBN (0.01–3 mg/kg). In an additional study, BN (2, 5, 10 or 20 mg/kg/hr) or NBN (2, 5, 10 or 15 mg/kg/hr) was infused continuously through a femoral vein cannula. Blood samples were collected through the femoral artery cannula at 2, 5, 15, 30, 60, 120, 240 and 480 min after administration. Before each sampling period, the respiratory rate (number of breaths per minute) was counted for 1 min, the count being based on the up-and-down movement of the abdomen caused by the animal’s breathing.

Interaction between NBN and opioid receptor antagonists. The effects of three opioid-receptor antagonists, naloxone, β-FNA and ICI 174864, on the respiratory depression induced by NBN were studied. NBN (3 mg/kg) was administered i.v. through the femoral venous cannula, and then naloxone (1 mg/kg), β-FNA (8 mg/kg) or ICI 174864 (4 mg/kg) was given through the same cannula 5 min after NBN administration. Respiratory rates were determined at appropriate time intervals until 480 min after the injection, as described above.

BN and NBN assays. The concentration of BN or NBN in plasma and tissue (brain, heart and lungs) was determined by a gas chromatographic-mass spectrometric method (Ohtani et al., 1989). Briefly, 0.05 M sulfuric acid (0.5 ml) and internal standard (25 ng) solution were added to plasma (0.5 ml) or tissue homogenate (0.5 ml). The aqueous phase was then washed with a mixture of ethyl acetate: n-heptane (4:1, v/v). After the pH of the aqueous phase had been adjusted to 10.5 with 1 M sodium carbonate-hydrochloric acid buffer solution, the analytes were extracted with the same organic solvent mixture. Derivatization was then performed using PFPA (50°C, 1 hr), and the fragment ions of PFP-BN (m/z 596), PFP-NBN (m/z 688) and PFP-internal standard (m/z 584) were monitored in a chemical ionization mode. A chromatographic column (1 m \times 2.6 mm i.D.) packed with 1% SE-52 Chromosorb W, AW-DMCS, 80 to 100 mesh, was used. The lower limits of the quantitation were 0.2 μg/ml from both plasma and tissue homogenates for both BN and NBN.

Pharmacokinetic analysis. The plasma concentration-time profiles of BN or NBN after i.v. administration were fitted to a biexponential equation using the nonlinear least-squares program MULTI (Yamaoka et al., 1978):

$$C_t = A e^{-a t} + B e^{-b t}$$

The terminal half-life ($T_{1/2}$), systemic clearance ($CL_s$), mean residence time (MRT) and steady-state volume of distribution ($V_{ds}$) were determined by conventional approaches (Gibaldi and Perrier, 1982). The area under the plasma concentration-time curve (AUC) for tracer amounts of BN or NBN was calculated by the linear trapezoidal rule.
was calculated by the linear trapezoidal method with extrapolation to infinity.

Statistical analysis. Statistical analysis was performed by paired analysis of variance with $P < 0.05$ as the minimum level of significance. All results are expressed as the mean ± S.D.

Results

Plasma level-time profiles, respiratory rate and arterial pCO$_2$ levels after i.v. bolus administration. The plasma concentration of BN declined biexponentially after administration of each dose of BN (fig. 1). The ranges of BN's mean pharmacokinetic parameters were as follows: $T_{1/2}$, 2.1 to 3.0 hr; $V_d$,$ss$, 3.2 to 4.4 l/kg and $CL_s$, 19 to 27 ml/kg/min. Neither the respiratory rate nor the arterial pCO$_2$ level changed over the dose range studied (0.008–3 mg/kg) (fig. 1). The plasma concentration of NBN after administration of NBN (0.01–3 mg/kg) also declined biexponentially with a $T_{1/2}$ of approximately 1 hr (fig. 2). The mean $V_d$,$ss$ and $CL_s$ values for NBN were 2.0 to 3.2 l/kg and 34 to 42 ml/kg/min, respectively. The respiratory rate decreased in a dose-dependent fashion; for example, the reduced rates after the doses of 1, 2 and 3 mg/kg were 12%, 22% and 70%, respectively, as compared with those of the control group of rats (fig. 2). An increase in the arterial pCO$_2$ levels was also noted, and it appeared to be related to the decrease in the respiratory rate (fig. 2).

Profiles of respiratory rates after constant i.v. infusion. The respiratory rate gradually decreased during i.v.
infusion of BN (20 mg/kg/hr) or NBN (2 mg/kg/hr), with a minimum rate being observed 3 and 1 hr after the beginning of the infusion, respectively. The relationships between infusion rate of BN or NBN and minimum respiratory rate are shown in figure 3. The reduction in the respiratory rate by BN was observed only at a high infusion rate (20 mg/kg/hr), and this decrease did attain statistical significance compared with control and the other BN administration groups. In contrast, NBN decreased the respiratory rate dose-dependently. On the basis of these results, we estimated the respiratory depressant activity of NBN to be approximately 10 times more potent than that of BN.

Profiles of plasma and brain drug concentration after i.v. and i.a. administration. Time courses of plasma and brain concentration of the drug after i.v. administration of BN or NBN are shown in figure 4. BN rapidly distributed into the brain, the concentration at 2 min after administration being the highest noted. The brain-to-plasma ratio of BN ranged from 1.1 to 3.3. By contrast, the brain distribution of NBN after its administration was much reduced when compared with that of the parent drug, and the peak concentration at 15 min after dosing was only 20 ng/ml. The brain-to-plasma ratio of NBN was less than 0.1.

Relationship between respiratory rate and NBN tissue concentration after i.v. and i.a. administration. As shown in figure 5, the respiratory rate was markedly reduced after i.v. administration of NBN (1, 2 and 3 mg/kg). In contrast, the respiratory rate was not affected after i.a. administration of the same dose of NBN. The plasma and tissue concentrations of NBN at 15 min after i.v. and i.a. administration of NBN (3 mg/kg) are summarized in table 1. The plasma, brain and heart concentrations of NBN after i.v. administration were almost the same as those after i.a. administration. However, the concentration in the lung after i.v. administration was approximately 4-fold higher.

Effect of opioid antagonists on respiratory rate. As shown in figure 6, the respiratory depression induced by NBN was immediately reduced after the administration of naloxone (opioid mu-, delta- and kappa-receptor antagonist) and β-FNA (a selective opioid mu-receptor antagonist) when these antagonists were given i.v. at 5 min after administration of NBN (3 mg/kg). However, the respiratory depression was not improved by ICI 174864 (a selective opioid delta-receptor antagonist).

Discussion

These studies clearly demonstrate that NBN, a metabolite of BN, produces dose-related respiratory depression and that, moreover, NBN is about 10 times more potent than the parent drug (figs. 2 and 3). The respiratory depression induced by the parent drug has been reported to occur in awake
or anesthetized humans and in conscious animals (Heel et al., 1979; Shook et al., 1990). Cowan et al. (1977) reported that the depression induced by BN developed at a dose of 0.01 mg/kg i.a. and reached a plateau (i.e., ceiling) over a dose range of 0.1 to 10 mg/kg i.a. in rats. However, in our study, when a small dose of BN (0.008 and 0.1 mg/kg) was administered i.a. or i.v., respiratory depression was not observed (fig. 1). The cause of this discrepancy is unclear. As shown in figure 2, the peak effect was seen at 15 min after administration of NBN. The mechanism of this delayed peak effect is not known. Willette and Sapru (1982) also reported that in decerebrate rats, although bradycardia, apnea and a transient biphasic blood pressure response were seen within 1 sec after administration of morphine into the right atrium, a delayed respiratory depressant action was observed after 8 min.

Although NBN has low permeability into the brain, this metabolite produced a marked disturbance in ventilation (fig. 4). Therefore, to study whether NBN-induced respiratory depression was mediated by the CNS, we further investigated the relationship between the respiratory depression and the pharmacokinetic behavior of NBN in the lung and brain after administration of NBN from two different routes (i.a. and i.v.) (fig. 5; table 1). The respiratory depression did not develop even at a high i.a. dose (3 mg/kg) of NBN, and the concentration of NBN in the lungs after i.v. administration was much higher than after i.a. administration. In spite of the similarity of the concentrations in the brain with the two routes, these results suggest that the respiratory depression induced by NBN may be mediated by opioid receptors in the lung rather than in the brain. The results of the i.v. and i.a. administration experiments also imply that a 3.5-fold larger i.a. dose should have a substantial effect on respiratory depression. Opioid receptors and enkephalins have been identified in peripheral mammalian tissues, including lung tissue (Hughes et al., 1977). Belvisi et al. (1990) demonstrated that both mu- and delta-opioid receptors, which are related to respiratory depression, are present in guinea pig bronchi. Sapru et al. (1981) reported that the stable analog D-met²-pro⁵-enkephalinamide, administered into the right atrium of decerebrate rats, immediately induced apnea followed by a period of rapid and shallow breathing. Willette and Sapru (1982) also reported that the administration of morphine sulfate into the right atrium of decerebrate rats produced the same effects as D-met²-pro⁵-enkephalinamide. These results suggest that the initial cardiorespiratory effects of morphine are due to a peripheral reflex action arising from the stimulation of opiate receptors associated with pulmonary C-fiber, e.g., J-fibers. Therefore, it is likely that NBN also causes respiratory depression by the same mechanism.

BN has been pharmacologically classified as a partial mu-agonist, but it has also been shown to have affinity at the delta and kappa sites (Villiger and Tailor, 1981; Wood and Rackham, 1982; Lewis, 1986). Lewis (1986) claimed that there is no report on the intrinsic activity of BN at kappa-receptors and that its kappa intrinsic activity may be low in order to account for the lack of dysphoric effects in humans. In general, the kappa-selective agonists appear to have little effect on ventilation, despite their relative lack of selectivity in receptor binding assays (Shook et al., 1990; Murphey and Olson, 1994). In contrast, the binding to the opioid receptor by NBN is still unclear. Accordingly, we performed experiments using three different opioid-receptor antagonists—naloxone, β-FNA and ICI 174864—to determine which specific opioid receptor(s) may be involved in the respiratory depression by BN. It has been reported that naloxone antagonizes mu- and delta-agonists (Pazos and Florez, 1984); β-FNA selectively and irreversibly antagonizes mu-agonists, but not kappa- and delta-agonists (Takemori et al., 1981; Ward et al., 1982) and ICI 174864 selectively antagonizes delta-agonists (Blazquez and Garzon, 1994). On the basis of the findings that NBN-induced respiratory depression was readily reduced by the administration of naloxone and β-FNA, but not ICI 174864, we suggest that the depressant may be mediated by the opioid mu-receptors. Among opioid mu-receptor subtypes, mu-2 receptor is involved mainly in respiratory depression, and mu-1 receptor in the analgesic action. Pharmacologically, NBN has a weaker analgesic activity than the parent drug (Ohtani et al., 1995) but has a more potent respiratory depression action. This difference in the action between the parent drug and the metabolite might be produced by the difference in the affinities for mu-1 and mu-2 receptor. Further research will be required to resolve this disparity.

To investigate the issue from a toxicodynamic viewpoint, we examined the analgesic and respiratory potencies by comparing the results of the present study with those of a previous report (Ohtani et al., 1995). As shown in figure 7, after BN administration, an analgesic effect is produced at low plasma BN concentration, whereas respiratory depression is not induced even at a 1000-fold higher concentration. By contrast, to obtain with NBN an analgesic effect comparable to that with BN, it is necessary to maintain the NBN concentration at a 50-fold higher value, at which point the concentration that induced respiratory depression is closer to that which produced the analgesic effect.

In conclusion, we found that the metabolite NBN is a respiratory depressant with considerably greater potency than that of the parent drug. Furthermore, the data suggest that NBN-induced respiratory depression may be primarily mediated by opioid receptors in the peripheral tissue, probably in the lung, rather than in the brain.
References


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