0022-3565/84/2302-0353$02.00/0
THE JOURNAL OF PHARMACOLOGY AND EXPERIMENTAL THERAPEUTICS
Vol. 230, No. 2

Printed in U.S.A.

Effects of Diazepam on Arterial Blood Gas Concentrations and pH of Adult Rats Acutely and Chronically Exposed to Methadone[1]

GWENDOLYN Y. McCORMICK,[2] WILLIAM J. WHITE, IAN S. ZAGON and C. MAX LANG

Departments of Comparative Medicine and Anatomy, The Milton S. Hershey Medical Center, The Pennsylvania State University, Hershey, Pennsylvania

Accepted for publication May 7, 1984

ABSTRACT

The effects of diazepam alone, and in combination with acute and chronic exposure to methadone, on arterial pH, pCO_2 and pO_2 in the rat were evaluated. Measurements were made before drug administration and at 15, 30, 60, 120, 180 and 240 min postadministration. Diazepam (20 mg/kg s.c.) did not cause any significant changes in arterial pCO_2 or pH. However, it did cause a significant increase in arterial pO_2 tension ($P \leq .05$). The magnitude of this effect was essentially the same after acute and chronic diazepam treatment. The increase in arterial pO_2 tension was attributed to a decrease in tissue uptake of oxygen associated with the decrease in body temperature that occurred after diazepam treatment. Acute and, to a far lesser extent, chronic administration of methadone (5 mg/kg/day i.p.) caused significant decreases in arterial pH and pO_2 and increases in pCO_2 ($P \leq .05$). When given in combination with methadone, diazepam potentiated markedly the respiratory depressant effects of methadone. The most severe respiratory depression occurred when both drugs were used together acutely. The effects of the acute diazepam-chronic methadone treatment were 100 to 200% greater than those that occurred with the chronic diazepam-chronic methadone treatment, indicating the development of a tolerance to the potentiating ability of diazepam. These results show that there is a real potential for severe respiratory depression when these drugs, methadone and diazepam, are used concurrently, especially for the first time.

Respiratory depression is a common adverse reaction associated with the use of methadone and other narcotics (Jaffe and Martin, 1980; Eckenhoff and Oech, 1960). It also is a reported side effect of diazepam and other members of the benzodiazepine group (Dixon *et al.*, 1970; Forster *et al.*, 1980; Divoll *et al.*, 1981). Whereas the respiratory depressant effects of therapeutic doses of narcotics can potentially be lethal to the narcotic-naive individual (Eckenhoff and Oech, 1960), the use of therapeutic doses of diazepam rarely causes clinical signs of respiratory embarrassment unless the individual has preexisting obstructive pulmonary disease (Delpierre *et al.*, 1981; Rao *et al.*, 1973; Soroker *et al.*, 1978).

Because both of these drugs have the potential for causing respiratory depression, it is possible that the combined use of these drugs could be very dangerous. Although therapeutic drug levels of methadone and diazepam have been found together in victims of multiple drug-related deaths in the U.S. and Canada (Finkle *et al.*, 1979; Lewis *et al.*, 1979), it has not been proven conclusively that they, or other drugs also found in those individuals, played significant roles in the deaths.

Today, this drug combination of methadone and diazepam is widely used by narcotic addicts in methadone treatment programs (Kleber and Gold, 1978; Stitzer *et al.*, 1981). Forty to seventy percent of participants have reported that they frequently use diazepam supplied by their supervising clinician or obtained it from illegal drug dealers (Dewy, 1972; Woody *et al.*, 1975a,b; Stitzer *et al.*, 1981).

Studies of the interaction of diazepam and methadone in humans have been hampered due to the difficulty in obtaining a representative group of subjects that is homogenous with regard to sex, age, physical condition and prior exposure to abused drugs. Hence the use of experimental animals in which these variables are more easily controlled provide an acceptable alternative. The laboratory rat has been shown to be susceptible to the central nervous system depression caused by either diazepam or methadone (Greenblatt and Shader, 1973; White and Zagon, 1979) and the pharmacokinetics of these drugs in humans and rats are well documented (Basel and Casarett, 1972; Schwartz *et al.*, 1967; Balderssarini, 1980; Jaffe and Martin, 1980).

Received for publication August 12, 1983.
[1] This work was supported in part by National Institutes of Health Grants RR00469 and RR07006 and National Institute on Drug Abuse Grant DA-01618.
[2] Present address: School of Veterinary Medicine, North Carolina State University, 4700 Hillsborough St., Raleigh, NC 27606.

ABBREVIATION: CPD, citric acid-sodium biphosphate-dextran-adenine anticoagulant.

It was the purpose of this study to document the effects of diazepam and methadone on arterial pH, pCO_2 and pO_2 in the rat and to evaluate the interaction of diazepam and methadone in regards to their combined effect on the respiratory system after acute and chronic administration. Pepelko's and Dixon's (1975) technique for monitoring blood gas concentration and pH in rats was utilized and quantitative assessments of arterial pCO_2, pO_2 and pH in adult male rats were used as criteria for evaluation.

Materials and Methods

Animals. Male Sprague-Dawley rats (Charles River Breeding Labs, Wilmington, MA) weighing 325 to 350 g upon arrival were used in this study. All rats were quarantined for 5 days before the start of experimentation. The animals were housed five per stainless-steel wire-bottom cage and kept in an environment maintained at 21 ± 0.5°C with 50 ± 20% relative humidity and a 100% fresh air exchange rate of 15 complete changes per hour. The photoperiod was maintained at 12 hr of light (7:00 A.M.–7:00 P.M.) and 12 hr of dark (7:00 P.M.–7:00 A.M.) without twilight. Rat chow from Agway, Inc. (Syracuse, NY) and water were provided *ad libitum*. The rats were assigned to groups using a table of random numbers. They were weighed and identified with tail markings.

Drugs and treatment regimens. Dosages for methadone hydrochloride (Eli Lilly Co., Indianapolis, IN) and diazepam (Hoffmann-La Roche, Inc., Nutley, NJ) that have been shown to cause a nonfatal central nervous system depression in the rat (Greenblatt and Shader, 1973; White and Zagon, 1979) were selected for this study. All doses of methadone were diluted up to 1 ml with sterile water. Methadone was administered i.p. All doses of diazepam were diluted up to 1 ml with a propylene glycol solution. The propylene glycol solution, used in commercial diazepam preparations, was prepared so as to yield a solution with the final composition of 40% propylene glycol, 10% ethyl alcohol, 1.5% benzyl alcohol, 4.88% sodium benzoate and 0.12% benzoic acid. Diazepam was administered s.c.

The study was conducted in two phases. In the first phase of the study the effects of acute administration of 20 mg/kg of diazepam and 5 mg/kg of methadone individually, and in combination, on the arterial pH and blood gas concentrations were studied. The data collected from these animals were compared to that of controls which were given the solvents of the two drugs, sterile water (1 ml i.p.) and 40% propylene glycol solution (1 ml s.c.). Thirty-two animals were used in this phase, eight being assigned to each treatment regimen.

In the second phase of the study the effects of acute and chronic administration of 20 mg/kg of diazepam to rats treated chronically with 5 mg/kg of methadone were examined. The rectal temperature as well as the arterial pH, pCO_2 and pO_2 were measured. Rats treated chronically were given their drugs once daily for 14 days. Those that received the acute diazepam-chronic methadone treatment were given one dose of diazepam on the 14th day of methadone treatment. The data collected from these rats were compared to that from rats receiving chronic methadone, chronic diazepam or the solvents of the drugs (sterile water and propylene glycol solution). Forty animals were used in this phase, eight being assigned to each treatment regimen.

Catheter implantation and sampling procedures. On the days designated for blood collection and analysis (day of acute administration of drugs in phase one and day 14 of chronic treatment in phase two), each rat was anesthetized with a halothane-oxygen mixture using an open anesthetic delivery system (Soma, 1971) before implantation of an arterial catheter. Total anesthesia time per animal was 20 min and each was allowed a 2-hr recovery period before further experimentation. This recovery period was selected because it has been shown that when most animals are exposed to halothane anesthesia for up to 1 hr the arterial blood gas concentrations and pH return to preanesthetic values within 2 hr after the administration of the gas has been discontinued (Fisher, 1961).

A Teflon catheter, pretreated with heparinized saline, was implanted surgically into the middle caudal tail artery using techniques described by Pepelko and Dixon (1975). The catheter was held in place by means of 3-0 suture and a gauze-adhesive tape bandage. The rats were placed in individual plastic restraint chambers for recovery and blood collection. Compressed air was blown into the restraint chambers through a plastic tube connected to the front of the restraint chamber to prevent carbon dioxide build-up within the chamber.

Immediately before the administration of the drug treatments and at 15, 30, 60, 120, 180 and 240 min after treatments, a 0.6-ml arterial blood sample was collected from each rat. Blood samples were analyzed for pH, pCO_2 and pO_2 content using an Instrumentation Laboratories blood gas analyzer (Boston, MA). After each sampling, 0.5 ml of CPD-treated blood and 0.5 ml of heparinized saline were infused into the tail artery of each rat to maintain blood volume and prevent the respiratory alkalosis associated with blood loss (Miller *et al.*, 1976). Heparinized saline was prepared by adding 3000 USP U of heparin sodium to 500 ml of sterile saline. CPD-treated blood was prepared by mixing 1 ml of the anticoagulant (*i.e.*, CPD) to each 10 ml of blood taken from a male Sprague-Dawley blood donor that had not received any drugs.

Rectal temperature. The rectal temperature of each rat was monitored before drug administration and at 30, 60, 120, 180 and 240 min postinjection of drug treatment in phase two. A Bailey Instruments electronic thermometer (Saddle Brook, NJ) was used to measure the rectal temperature in degrees centigrade.

Statistical analysis. A two-way analysis of variance was used to evaluate the blood gas concentration and pH data collected in each phase of the experiment. A three-way analysis of variance was used to compare the results obtained in phases one and two. Length of treatment (acute and chronic) and type of drug treatment were treated as between group factors, whereas sampling time was treated as a repeated measure. Further analysis between the drug-exposed groups and controls within each phase of the experiment matched for time, as well as additional comparisons between postinjection time periods and the preinjection time periods, were made using the Newman-Keuls procedure (Winer, 1971). A probability of .05 or less was considered significant.

Results

Phase I

Behavior. Control animals treated acutely with sterile water and propylene glycol remained alert during the entire testing period. Animals that were given 5 mg/kg of methadone were sleeping, but could be aroused by tapping on the top of the restraint chamber at 15 and 30 min postinjection and were completely alert at 60 min postinjection. Rats that received 20 mg/kg of diazepam were in a deep sleep at the 15-min sampling period and were not completely awake until 240 min postinjection. Rats that were injected with both 20 mg/kg of diazepam and 5 mg/kg of methadone were in a deep sleep in less than 5 min and did not become completely alert until 240 min postinjection.

pH. There were significant differences ($F = 13.92$, $dF = 3/28$, $P \leq .05$) between the mean arterial pH values of the groups tested. Data for these groups are presented in figure 1. Although the administration of 20 mg/kg of diazepam failed to elicit a detectable change in pH, when given in combination with methadone a 166% greater drop in arterial pH occurred than when methadone was given alone.

pCO_2. The analysis of pCO_2 values revealed a significant difference between the treatment groups ($F = 13.77$, $dF = 3/28$, $P \leq .05$). The data for these groups are presented in figure 2. Whereas the arterial pCO_2 of the controls and rats receiving diazepam injections remained essentially unaltered during the 240-min study, rats given methadone had a postinjection ele-

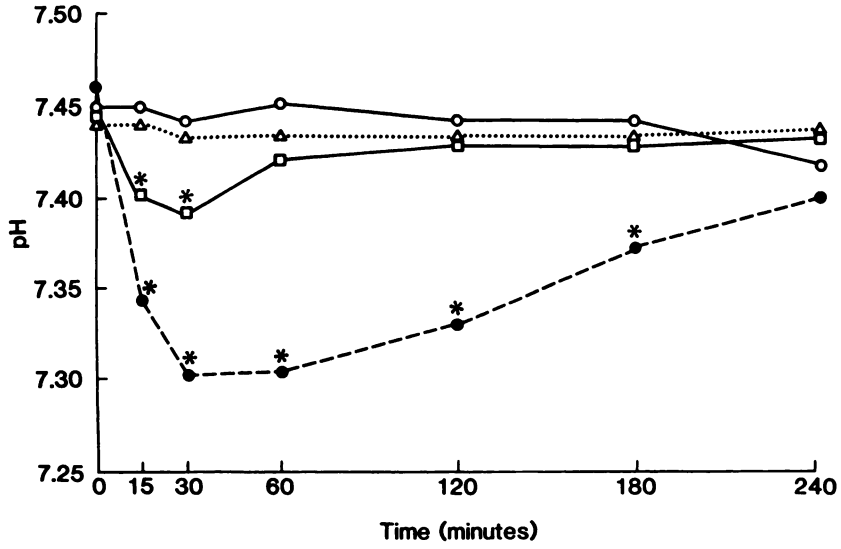

Fig. 1. Effect of acute exposure to diazepam and methadone on arterial pH in rats. Values represent mean blood pH for eight adult male rats at each postinjection time interval. S.E.s < 1% of mean values. Drugs were administered after the 0 time reading. *Significantly different from control at matched time intervals ($P < .05$). O, 1 ml of sterile water (i.p.) and 1 ml of 40% propylene glycol solution (s.c.); Δ, 20 mg/kg of diazepam (s.c.); □, 5 mg/kg of methadone (i.p.); ●, 20 mg/kg of diazepam (s.c.) and 5 mg/kg of methadone (i.p.).

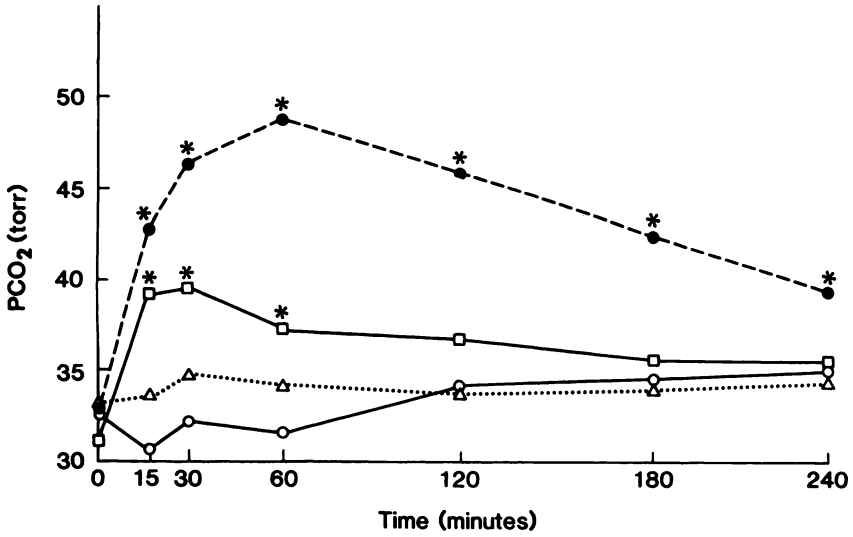

Fig. 2. Effect of acute exposure to diazepam and methadone on arterial pCO_2 in rats. Values represent mean blood pCO_2 for eight adult male rats at each postinjection time interval. S.E.s < 7% of mean values. Drugs were administered after the 0 time reading. *Significantly different from control at matched time intervals ($P < .05$). O, 1 ml of sterile water (i.p.) and 1 ml of 40% propylene glycol solution (s.c.); Δ, 20 mg/kg of diazepam (s.c.); □, 5 mg/kg of methadone (i.p.); ●, 20 mg/kg of diazepam (s.c.) and 5 mg/kg of methadone (i.p.).

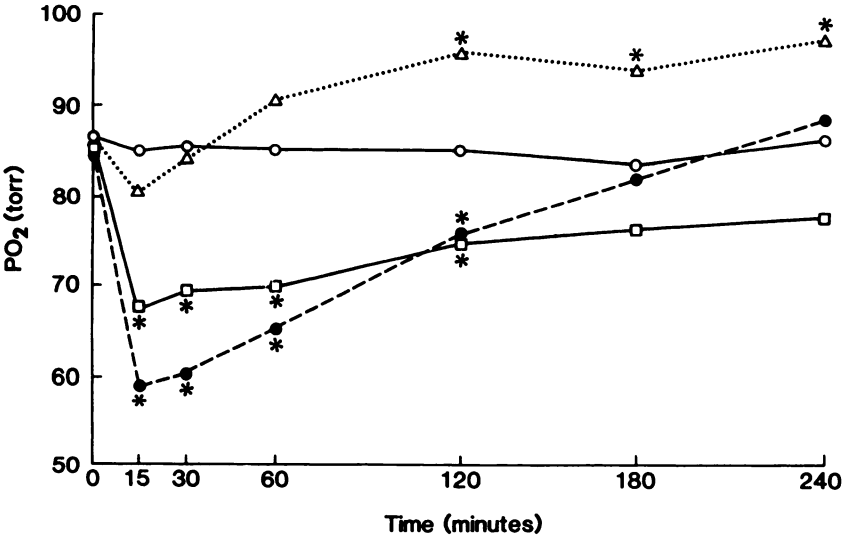

Fig. 3. Effect of acute exposure to diazepam and methadone on arterial pO_2 in rats. Values represent mean blood pO_2 for eight adult male rats at each postinjection time interval. S.E.s < 9% of mean values. *Significantly different from control at matched time intervals ($P < .05$). O, 1 ml of sterile water (i.p.) and 1 ml of 40% propylene glycol solution (s.c.); Δ, 20 mg/kg of diazepam (s.c.); □, 5 mg/kg of methadone (i.p.); ●, 20 mg/kg of diazepam (s.c.) and 5 mg/kg of methadone (i.p.).

vation of pCO_2 which was 20% higher than their preinjection values at the 30-min sampling period. An even greater elevation of arterial pCO_2 was observed in those rats given both diazepam and methadone, with a maximum elevation of 53% above preinjection values occurring at the 60-min sampling period.

pO_2. The analysis of pO_2 values revealed a significant difference between the treatment groups ($F = 8.89$, dF = 3/28, $P \leq .05$). The data for these treatment groups are presented in figure 3. When rats were given only diazepam, no remarkable changes were observed in their arterial pO_2 within the first 60 min postinjection. However, after that time period the mean arterial pO_2 of the diazepam group increased with time such that for the remainder of the test period the mean pO_2 was 14% higher than the preinjection value. In contrast, the rats

that were given methadone or methadone and diazepam had 21 and 30% decreases in arterial pO_2, respectively, within the first 15 min postinjection. Although neither of these two groups had a pO_2 that was significantly higher than the control, by the 240-min period the arterial pO_2 of the methadone-diazepam group was 17% higher than that of the methadone group.

Phase II

Behavior. Rats which received sterile water and propylene glycol or methadone chronically always remained alert after their injections. Those that were treated with diazepam chronically were in a deep sleep 15 min postinjection, arousable at 180 min and fully awake at 240 min postinjection. After administration of diazepam acutely or chronically in conjunction with chronic methadone treatment, the rats were in a deep sleep in less than 5 min postinjection, sleeping but arousable by 180 min and fully awake by 240 min postinjection.

pH. There were significant differences in arterial pH values between the treatment groups ($F = 24.53$, $dF = 4/35$, $P \leq .05$). The data for these treatment groups are presented in figure 4. Whereas there were no significant changes in the arterial pH of rats treated chronically with diazepam, rats that were given both diazepam and methadone chronically had a decrease in pH that was 50% lower than the decrease observed in those treated chronically with only methadone. An even greater decrease in pH occurred when an acute dose of diazepam was given to rats that had been treated chronically with methadone. At 30 min postinjection, the mean arterial pH of that group reached a maximum low which was three times the drop in pH observed in the chronic methadone group.

pCO_2. In the analysis of pCO_2 values, there was a significant difference between the groups ($F = 7.93$, $dF = 4/35$, $P \leq .05$). The data for these groups are presented in figure 5. The 20-mg/kg dose of diazepam administered chronically produced no detectable pCO_2 changes, yet when it was given acutely or chronically to rats treated chronically with methadone, a 29 and 19% increase in arterial pCO_2, respectively, above control values was noted. These changes were 81 and 19%, respectively, greater than changes elicited by chronic methadone injections alone.

pO_2. There was a significant difference in pO_2 values between the treatment groups ($F = 9.86$, $dF = 4/35$, $P \leq .05$). The data for these groups are presented in figure 6.

In contrast to the chronic diazepam group that had no significant changes in arterial pO_2 during the first 30 min postinjection, the chronic methadone-chronic diazepam, chronic methadone and chronic methadone-acute diazepam groups had decreases in arterial pO_2 within the first 15 min postinjection that were 14, 12 and 8%, respectively, lower than controls.

After 60 min postinjection, a significant elevation of arterial pO_2 occurred in all groups receiving diazepam acutely or chronically, with or without methadone. At the 240-min period the pO_2 of these groups was 16 to 21% higher than controls, whereas the chronic methadone group did not differ significantly from the control at that time.

Rectal temperature. There was a significant difference in rectal temperature of the treatment groups ($F = 7.27$, $dF = 4/35$, $P \leq .05$). As illustrated in figure 7, the rectal temperature of rats receiving diazepam alone or in combination with methadone decreased to a point significantly lower than the controls from 60 to 240 min postinjection of the drugs.

Discussion

The acute and chronic administration of 20 mg/kg of diazepam failed to induce any detectable changes in the arterial pH and pCO_2 in the rat. This is at variance with observations seen in humans (Gross *et al.*, 1982) and rabbits (Bradshaw *et al.*, 1973), in which healthy subjects given 0.4 mg/kg and 4 mg/kg, respectively, experienced significant changes in these parameters. This may be the result of a species variation in resistance to the respiratory depressant effects of diazepam, as there are also differences in doses that are considered lethal for these species: humans, LD_{Lo} = 50 mg/kg; rabbit, LD_{50} = 900 mg/kg; rat, LD_{50} = 300 mg/kg (U.S. Department of H.E.W. *et al.*, 1977). A much higher dosage would perhaps be required to elicit detectable respiratory depression in the rat.

The observation that the dose of diazepam that caused sedation in the rat failed to cause an alteration in arterial pCO_2 and pH is in contrast to observations in humans (Gross *et al.*, 1982). Gross and his associates (1982) have suggested that there is a strong correlation between the degree of respiratory depression and the state of consciousness after diazepam administration, as humans given 0.4 mg/kg of diazepam i.v. experienced both peak respiratory depression and unconsciousness within the first 30 min postinjection. However, the observations made in this study show that there is not a correlation between the state of consciousness and the arterial blood gases and pH after diazepam treatment.

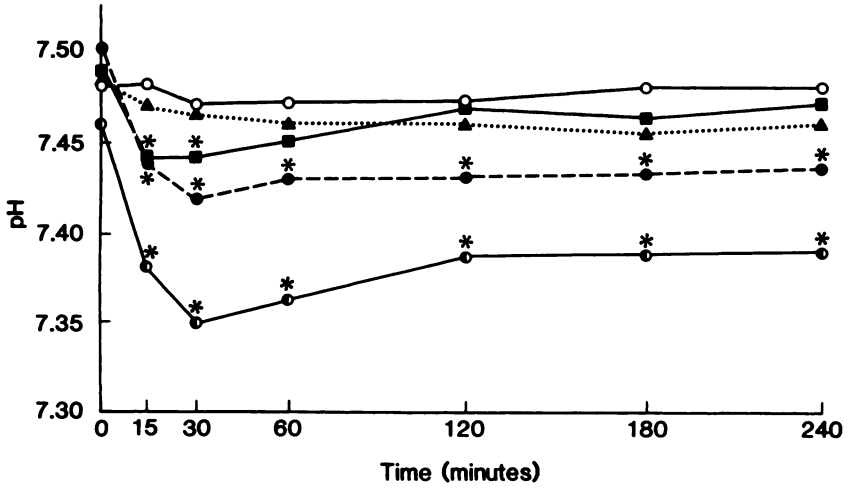

Fig. 4. Effects of acute and chronic exposure to diazepam on arterial pH of rats chronically exposed to methadone. Values represent mean blood pH for eight adult male rats at each postinjection time interval. S.E.s < 1% of mean values. Drugs were administered after the 0 time reading. *Significantly different from controls at matched time intervals ($P < .05$). ○, 1 ml of sterile water (i.p.) and 1 ml of 40% propylene glycol solution (s.c.) chronically; ▲, 20 mg/kg of diazepam (s.c.) chronically; ■, 5 mg/kg of methadone (i.p.) chronically; ●, 20 mg/kg of diazepam (s.c.) and 5 mg/kg of methadone (i.p.) chronically; ◐, 5 mg/kg of methadone (i.p.) chronically and 20 mg/kg of diazepam (s.c.) acutely.

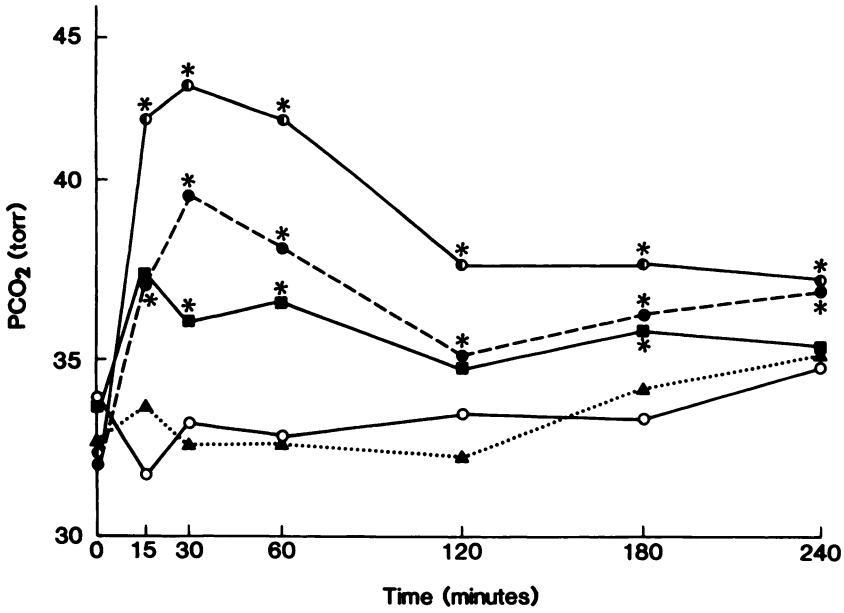

Fig. 5. Effects of acute and chronic exposure to diazepam on arterial pCO_2 of rats chronically exposed to methadone. Values represent mean blood pCO_2 for eight adult male rats at each postinjection time interval. S.E.s < 6% of mean values. Drugs were administered after the 0 time reading. *Significantly different from controls at matched time intervals ($P < .05$). ○, 1 ml of sterile water (i.p.) and 1 ml of 40% propylene glycol solution (s.c.) chronically; ▲, 20 mg/kg of diazepam (s.c.) chronically; ■, 5 mg/kg of methadone (i.p.) chronically; ●, 20 mg/kg of diazepam (s.c.) and 5 mg/kg of methadone (i.p.) chronically; ◐, 5 mg/kg of methadone (i.p.) chronically and 20 mg/kg of diazepam (s.c.) acutely.

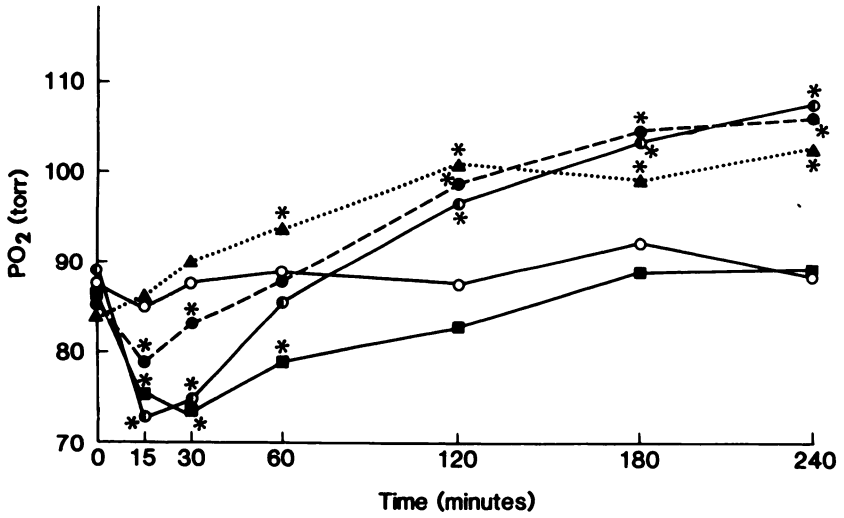

Fig. 6. Effects of acute and chronic exposure to diazepam on arterial pO_2 of rats chronically exposed to methadone. Values represent mean blood pO_2 for eight adult male rats at each postinjection time interval. S.E.s < 7% of mean values. Drugs were administered after the 0 time reading. *Significantly different from controls at matched time intervals ($P < .05$). ○, 1 ml of sterile water (i.p.) and 1 ml of 40% propylene glycol solution (s.c.) chronically; ▲, 20 mg/kg of diazepam (s.c.) chronically; ■, 5 mg/kg of methadone (i.p.) chronically; ●, 20 mg/kg of diazepam (s.c.) and 5 mg/kg of methadone (i.p.) chronically; ◐, 5 mg/kg of methadone (i.p.) chronically and 20 mg/kg of diazepam (s.c.) acutely.

In contrast to its lack of effect on arterial pCO_2 and pH, the 20-mg/kg dose of diazepam did cause an increase in arterial oxygen tension when given acutely or chronically. An increase in arterial pO_2 after diazepam treatment has not been reported to occur in humans. However, in most of these clinical studies only arterial pH and pCO_2 were monitored (Catchlove and Kafer, 1971; Gasser and Bellville, 1976; Gross *et al.*, 1982). In those few studies in which arterial pO_2 was monitored (Dixon *et al.*, 1970; Rao *et al.*, 1973), no measurements were made after 30 min postinjection, which was the time after which the rise in pO_2 was observed in the rats. An increase in arterial pO_2 has been reported in dogs treated acutely with 3 mg/kg of diazepam and chronically with 10 mg/kg of diazepam (Stepanek, 1973). Stepanek (1973) suggested that this was due to a diazepam-induced decreased tissue oxygen consumption after acute treatment and a compensatory mechanism to correct a metabolic acidosis associated with the accumulation of diazepam breakdown products after chronic use. A metabolic acidosis was not associated with chronic use of diazepam in this study; however, there is evidence that suggests a decrease in tissue uptake of oxygen may have occurred after diazepam treatment. Animals treated with diazepam alone or in combination with methadone had a decrease in rectal temperature that was significantly lower than that of controls from 60 to 240 min postinjection. Blair (1971) has shown that as the body temperature decreases from 37 to 30°C there is a dramatic decrease in cellular oxygen uptake. This is very likely the basis of the increased arterial pO_2 tension observed in the rats after diazepam treatment in this study.

Although the 20-mg/kg dose of diazepam did not elicit detectable respiratory depression when given alone, it was able to potentiate the respiratory depression caused by methadone. When the two drugs were given together acutely, the arterial pCO_2 tension increased by 45% and the pO_2 decreased by 33% compared to an increase of arterial pCO_2 of only 19% and a reduction of arterial pO_2 of only 16% when the methadone was used alone. These results corroborate those of Bradshaw *et al.* (1973) that showed potentiation of the morphine-induced elevation of arterial pCO_2 when diazepam and morphine were used together acutely.

The results of this study also show that the respiratory depressant effects of methadone and the ability of diazepam to potentiate this effect decreases with chronic use. The effects produced by the acute diazepam-acute methadone treatment

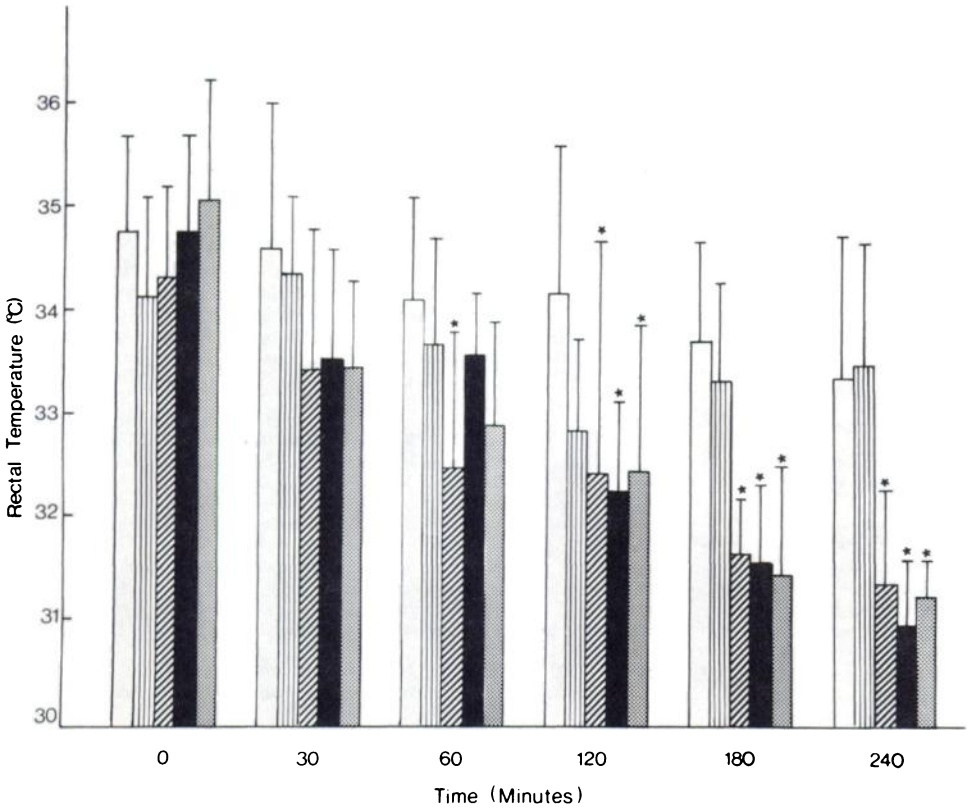

Fig. 7. Effects of acute and chronic exposure to diazepam on rectal temperature of rats chronically exposed to methadone. Values represent the mean rectal temperature ± S.D. (vertical lines) for eight adult male rats at each postinjection time interval. Drugs were administered after the 0 time reading. *Significantly different from control at matched time intervals ($P < .05$). □, 1 ml of sterile water (i.p.) and 1 ml of 40% propylene glycol solution (s.c.) chronically; ■, 5 mg/kg of methadone (i.p.) chronically; ▨, 20 mg/kg of diazepam (s.c.) chronically; ■, 20 mg/kg of diazepam (s.c.) and 5 mg/kg of methadone (i.p.) chronically; □, 5 mg/kg of methadone (i.p.) chronically and 20 mg/kg of diazepam (s.c.) acutely.

were 100 to 200% greater than that caused by the acute diazepam-chronic methadone treatment, which was in turn 100 to 200% greater than caused by the chronic diazepam-chronic methadone treatment. This decrease in respiratory depression observed with chronic use of the drugs is probably related to the development of receptor tolerance as has been reported to occur with chronic use of other opioids (Simon and Hiller, 1978) and benzodiazepines (Al-Khudhairi *et al.*, 1982; Barnett and Fiore, 1973; Braestrup and Olsen, 1980; Lippa *et al.*, 1982).

The development of a tolerance to the ability of diazepam to potentiate the respiratory depressant effect of methadone is in contrast to what was observed in its effect on sedation and arterial pO_2 tension. The magnitude of the effect of diazepam on these parameters, as monitored in this study, were essentially the same after acute and chronic treatment. This suggests that diazepam interacts with different receptor types to elicit its effects. This viewpoint has been proposed by several other investigators as well (Gee and Yamamura, 1982; Lippa *et al.*, 1982).

Because diazepam and methadone interact with different receptor types to cause their effects on the respiratory system (Gee and Yamamura, 1982; Jaffe and Martin, 1980; Mohler and Okada, 1977; Ward and Holaday, 1982), yet compete for metabolism by the same hepatic enzyme system (Spaulding *et al.*, 1974), the potential for severe respiratory depression is great when the two drugs are used concurrently. As shown in this study, when diazepam and methadone were used at dosages less than one-tenth of the LD_{50} for these drugs in the rat (U.S. Department of H.E.W. *et al.*, 1977, 1979), significant respiratory depression occurred. Because this potential exists, great caution should be taken when these drugs are used concurrently.

References

AL-KHUDHAIRI, D., ASKITOPOULOU, H. AND WHITMAN, G.: Acute "tolerances" to the central respiratory effects of midazolam in the dog. Br. J. Anaesth. **54:** 953–958, 1982.

BALDERSSARINI, R. J.: Drugs and the treatment of psychiatric disorders. *In* The Pharmacological Basis of Therapeutics, ed. by A. G. Gilman, L. S. Goodman and A. Gilman, pp. 391–447, The MacMillan Company, New York, 1980.

BARNETT, A. AND FIORE, J. W.: Acute tolerance to diazepam in cats. In The Benzodiazepines, ed. by S. Garratini, E. Mussini and L. O. Randall, p. 545, Raven Press, New York, 1973.

BASELT, R. C. AND CASARETT, L. J.: Biliary and urinary elimination of methadone and its metabolites in the rat. Biochem. Pharmacol. **21:** 2705–2712, 1972.

BLAIR, E.: Hypothermia. *In* Textbook of Veterinary Anesthesia, ed. by L. R. Soma, pp. 555–579, The Williams and Wilkins Company, Baltimore, 1971.

BRADSHAW, E. G., BISWAS, T. K. AND PLEUVRY, B. J.: Some interactions between morphine and diazepam in the mouse and the rabbit. Br. J. Anaesth. **45:** 1185–1190, 1973.

BRAESTRUP, C. AND OLSEN, C. E.: Urinary and β-brain-carboline-3-carboxylates as potent inhibitors of brain benzodiazepine receptors. Proc. Natl. Acad. Sci. U.S.A. **77:** 2288–2292, 1980.

CATCHLOVE, R. F. H. AND KAFER, E. R.: The effects of diazepam on the ventilatory response to carbon dioxide and on steady-state gas exchange. Anesthesiology **34:** 9–13, 1971.

DELPIERRE, S., JAMMES, Y., GRIMAUD, C., DUGUE, P., ARNAUD, A. AND CHARPIN, J.: Influence of anxiolytic drugs (prazepam and diazepam) on respiratory center output and CO_2 chemosensitivity in patients with lung disease. Respiration **42:** 15–20, 1981.

DEWY, W. L.: Diazepam use of patients in methadone maintenance treatment programs. *In* Chemical and Biological Aspects of Drug Dependence, ed. by S. J. Mule and H. Brill, pp. 31–37, CRC Press, Cleveland, 1972.

DIXON, H. R., TILTON, B. E. AND BRIGGS, B. D.: A comparison of the sedative and cardiorespiratory effects of diazepam and pentazocine for premedication. Anesth. Analg. **49:** 546–550, 1970.

DIVOLL, M., GREENBLATT, D. J., LACASSE, Y. AND SHADER, R. I.: Benzodiazepine overdosage: Plasma concentrations and clinical outcome. Psychopharmacology **73:** 381–383, 1981.

ECKENHOFF, J. E. AND OECH, S. R.: The effects of narcotics and antagonists upon respiration and circulation in man: A review. Clin. Pharmacol. Ther. **1:** 488–524, 1960.

FINKLE, B. S., MCCLOSKEY, K. L. AND GOODMAN, L. S.: Diazepam and drug-associated deaths, a survey in the United States and Canada. J. Am. Med. Assoc. **242:** 429–434, 1979.

FISHER, E. W.: Observations on the disturbances of respiration of cattle, horses, sheep and dogs caused by halothane anesthesia and the change taking place in plasma pH and plasma CO_2 content. Am. J. Vet. Res. **22:** 279–286, 1961.

FORSTER, A., GARDEZ, J. P., SUTER, P. M. AND GEMPERLE, M.: Respiratory depression caused by midazolam and diazepam. Anesthesiology **53:** 494–497, 1980.

GASSER, J. C. AND BELLVILLE, J. W.: The respiratory effects of hydroxyzine, diazepam and pentazocine in man. Anesthesia **31:** 718–723, 1976.

GEE, K. AND YAMAMURA, H. J.: Regional heterogenicity of benzodiazepine receptors at 37°C: An *in vitro* study in various regions of the rat brain. Life Sci. **31:** 1939–1945, 1982.

GREENBLATT, D. J. AND R. I. SHADER: Benzodiazepines in Clinical Practice, Raven Press, New York, 1973.

GROSS, J. B., SMITH, L. AND SMITH, T. C.: Time course ventilatory response to carbon dioxide after diazepam. Anesthesiology **57:** 18–21, 1982.

JAFFE, J. H. AND MARTIN, W. R.: Opioid analgesics and antagonists. *In* The Pharmacological Basis of Therapeutics, ed. by A. G. Gilman, L. S. Goodman and A. Gilman, pp. 495–534, The MacMillan Company, New York, 1980.

KLEBER, H. D. AND GOLD, M. S.: Use of psychotropic drugs in treatment of methadone maintained narcotic addicts. *In* Recent Developments in Chemotherapy of Narcotic Addiction, ed. by B. Kissin, J. H. Lowinson and R. B. Millman, pp. 81–97, The New York Academy of Science, New York, 1978.

LEWIS, A. J., GONZALES, G. D. AND WINEK, C. L.: Modern Drug Encyclopedia, Technical Publishing Company, New York, 1979.

LIPPA, A. S., MEYERSON, L. AND BEER, B.: Molecular substrates of anxiety: Clues from the heterogeneity of benzodiazepine receptors. Life Sci. **31:** 1409–1417, 1982.

MILLER, M. E., RORTH, M., STOHLMAN, F., VALERI, C. R., LOWEIE, G., HOWARD, D. AND MCGILVRAY, N.: The effects of acute bleeding on acid-base balance, erythropoietin (EP) production and *in vivo* LD_{50} in the rat. Br. J. Haematol. **33:** 379–385, 1976.

MOHLER, H. AND OKADA, T.: Benzodiazepine receptors: Demonstration in the central nervous system. Science (Wash. DC) **198:** 849–851, 1977.

PEPELKO, W. E. AND DIXON, G. A.: Arterial blood gases in conscious rats exposed to hypoxia, hypercapnia, or both. J. Appl. Physiol. **38:** 581–587, 1975.

RAO, S., SHERBANIUK, R. W., PRASAD, K., LEE, S. J. K. AND SPROULE, B. J.: Cardiopulmonary effects of diazepam. Clin. Pharmacol. Ther. **14:** 182–189, 1973.

SCHWARTZ, M. A., BOMMER, P. AND VANE, F. M.: Diazepam metabolites in the rat: Characterization by high-resolution mass spectrometry and nuclear magnetic resonance. Arch. Biochem. Biophys. **121:** 508–516, 1967.

SIMON, E. J. AND HILLER, J. M.: The opiate receptors. Annu. Rev. Pharmacol. Toxicol. **18:** 371–394, 1978.

SOMA, L. R.: Systems and techniques for inhalation anesthesia. *In* Textbook of Veterinary Anesthesia, ed. by L. R. Soma, pp. 201–228, The Williams and Wilkins Company, Baltimore, 1971.

SOROKER, D., BARZILAY, E., KONICHEZKY, S. AND BRUDERMAN, I.: Respiratory function following premedication with droperidol or diazepam. Anesth. Analg. **57:** 695–699, 1978.

SPAULDING, T. C., MINIUM, L., KOTAKE, A. N. AND TAKEM, A. E.: The effects of diazepam on the metabolism of methadone by the liver of methadone-dependent rats. Drug Metab. Dispos. **2:** 458–463, 1974.

STEPANEK, J.: Changes in acid-base balance, respiration rate and heart rate upon repeated oral administration of benzoctamine and diazepam to conscious dogs. Arch. Int. Pharmacodyn. Ther. **202:** 135–152, 1973.

STITZER, M. L., GRIFFITHS, R. R., MCLELLAN, A. T., GRABOWSKI, J. AND HAWTHORNE, J. W.: Diazepam use among methadone maintenance patients: Patterns and dosages. Drug Alcohol Depend. **8:** 189–199, 1981.

U.S. DEPARTMENT OF HEALTH, EDUCATION AND WELFARE, PUBLIC HEALTH SERVICE, CENTER FOR DISEASE CONTROL, NATIONAL INSTITUTE FOR OCCUPATIONAL SAFETY AND HEALTH: Registry of Toxic Effects of Chemical Substances, 1976 edition, U.S. Government Printing Office, Washington, DC, 1977.

U.S. DEPARTMENT OF HEALTH, EDUCATION AND WELFARE, PUBLIC HEALTH SERVICE, CENTER FOR DISEASE CONTROL, NATIONAL INSTITUTE FOR OCCUPATIONAL SAFETY AND HEALTH: Registry of Toxic Effects of Chemical Substances, 1978 edition, U.S. Government Printing Office, Washington, DC, 1979.

WARD, S. J. AND HOLADAY, J. W.: Relative involvement of μ and σ opioid mechanisms in morphine induced depression of respiration in rats. Proc. Soc. Neurosci. **8:** 388, 1982.

WHITE, W. J. AND ZAGON, I. S.: Acute and chronic methadone exposure in adult rats: Studies on arterial blood gas concentrations and pH. J. Pharmacol. Exp. Ther. **209:** 451–455, 1979.

WINER, B. J.: Statistical Principles in Experimental Design, 2nd ed., McGraw-Hill Book Company, New York, 1971.

WOODY, G. E., MINTZ, T. AND O'HARE, K.: Diazepam use by patients in a methadone program—How serious a problem? J. Psychedelic Drugs **7:** 373–379, 1975a.

WOODY, G. E., O'BRIEN, C. P. AND GREENSTEIN, R.: Misuse and abuse of diazepam: An increasingly common medical problem. Int. J. Addict. **10:** 843–848, 1975b.

Send reprint requests to: Gwendolyn Y. McCormick, School of Veterinary Medicine, North Carolina State University, 4700 Hillsborough St., Raleigh, NC 27606.