Anesthesiology
60:537–540, 1984

Effects of Morphine and Respiratory Depression on Sulfobromophthalein Disposition in Rats

Aryeh Hurwitz, M.D., and Holly R. Fischer, B.G.S.†*

Morphine, 20 mg·kg^{-1}, sc, halved the plasma clearance of sulfobromophthalein (BSP) while tripling hepatic tissue levels of this dye. Since narcotics depress respiration, effects of hypoxia, hypercapnia, and acidosis on BSP disposition were studied. Ambient gases breathed by rats were adjusted to achieve blood gas levels identical to those of morphine-induced respiratory depression. Saline-treated rats breathing room air had PA_{O_2} of 87 ± 3 mmHg (mean ± SE) and Pa_{CO_2} of 40 ± 2 mmHg. After intraarterial injection of BSP, 100 mg·kg^{-1}, plasma clearance of this dye was 7.1 ± 1.1 ml·min^{-1} and BSP levels in the liver at 40 min after injection were 163.3 ± 19.8 μg·g^{-1}. After morphine, 20 mg·kg^{-1}, Pa_{O_2} decreased to 47 ± 4 mmHg and Pa_{CO_2} increased to 89 ± 5 mmHg. In these rats BSP clearance dropped to 3.5 ± 0.4 ml·min^{-1}, and 40-min liver dye levels were increased to 596.4 ± 60.4 μg·g^{-1}. Similar hypoxia and hypercapnia caused by breathing 9% O_2 and 8% CO_2 in the absence of morphine caused plasma BSP clearance to be decreased to 4.4 ± 0.2 ml·min^{-1} and 40-min hepatic BSP to be increased to 292.5 ± 31.8 μg·g^{-1}. Hypercapnia and acidosis alone did not affect BSP disposition, while hypoxia without hypercapnia decreased its plasma clearance to 5.5 ± 0.3 ml·min^{-1} and increased liver levels to 339.1 ± 35.1 μg·g^{-1}. Hypoxia was reversed completely in morphine-treated rats by placing them in 40% O_2. In these animals, despite normal oxygen, plasma BSP clearance was decreased to 4.4 ± 0.6 ml·min^{-1}, and liver BSP was increased to 497.9 ± 65.6 μg·g^{-1}. Thus, respiratory depression with hypoxia may contribute to morphine-induced effects on BSP disposition, but altered blood gases cannot account fully for these narcotic effects. (Key words: Acid–base equilibrium: acidosis, respiratory. Analgesics: morphine. Carbon dioxide: hypercarbia. Hypoxia. Liver: function; sulfobromophthalein.

NARCOTIC AGENTS are used widely in many clinical settings for relief of pain and, at higher doses, for anesthesia. While their effects on biliary function have been studied for many years, narcotic effects on compounds excreted into bile have been examined only recently.[1] Morphine slows the hepatobiliary elimination of the anionic dye, sulfobromophthalein (BSP), in rodents. This dye has been used extensively as a model for studying hepatobiliary function, and its disposition in rats is well known. Many of the known effects of narcotics could modify BSP disposition. Among these narcotic effects are respiratory depression, which results in hypoxia and carbon dioxide retention. The present study was designed to determine if the effects of morphine on BSP disposition were secondary to these blood gas changes from respiratory depression.

Methods

Male Holtzman-derived Sprague–Dawley rats (Sasco Farms, Omaha, Nebraska), weighing 300 to 400 g, were studied. On the morning before each experiment, cannulae (PE 50) were placed in the right carotid artery under light ether anesthesia and exteriorized through the skin in the back of the neck. Cannula patency was maintained by flushing with heparinized saline (100 IU/ml). Animals were given food and water *ad libitum* until the morning of the day of drug treatment. The animals were placed in a plastic restraining cage, which was flushed with the indicated mixture of oxygen and carbon dioxide, with the rest being nitrogen. These gases were bubbled through a water trap to assure adequate humidity. After equilibration for 3–4 h, saline or morphine sulfate, 20 mg·kg^{-1}, was given subcutaneously. Thirty minutes later, sodium BSP, 100 mg·kg^{-1}, was given intraarterially and the cannulae flushed with heparinized saline. Arterial blood samples were taken at 1, 2, 3, 4, 8, 12, 16, and 28 min for BSP determination and at 40 min for BSP and blood gas levels. Throughout the experiments, body temperature was maintained at 37°C by heating the cages. The animals then were killed with an intraarterial injection of T-61 Euthanasia Solution® (Hoechst, Somerville, New Jersey) and their livers removed.

Six groups of rats were studied. The 12 rats in Group I received saline (control) and breathed room air. The 13 rats in Group V also breathed room air but were given subcutaneous morphine sulfate, 20 mg·kg^{-1}. The resultant hypoxia and hypercarbia in these animals with morphine-induced respiratory depression were variously matched in the remaining groups by adjusting the gas mixtures breathed by saline-treated rats. The sequence of gas and morphine treatments was randomized so that rats in each of the six groups were studied at the same time. Group II (eight rats) were made hypoxic by breathing 11% O_2 and 89% N_2. The 12 rats in Group III breathed 8% CO_2 and 19% O_2, causing hypercarbia. Hy-

* Professor of Medicine and Pharmacology.

† Research Associate.

Received from the Department of Medicine, University of Kansas Medical Center, Kansas City, Kansas. Accepted for publication November 9, 1983. Supported by USPHS Grant DA 02477.

Address reprint requests to Dr. Hurwitz: Division of Clinical Pharmacology, the University of Kansas, 39th and Rainbow Boulevard, Kansas City, Kansas 66103.

Downloaded from http://asa2.silverchair.com/anesthesiology/article-pdf/60/6/537/308628/0000542-198406000-00003.pdf by guest on 17 April 2024

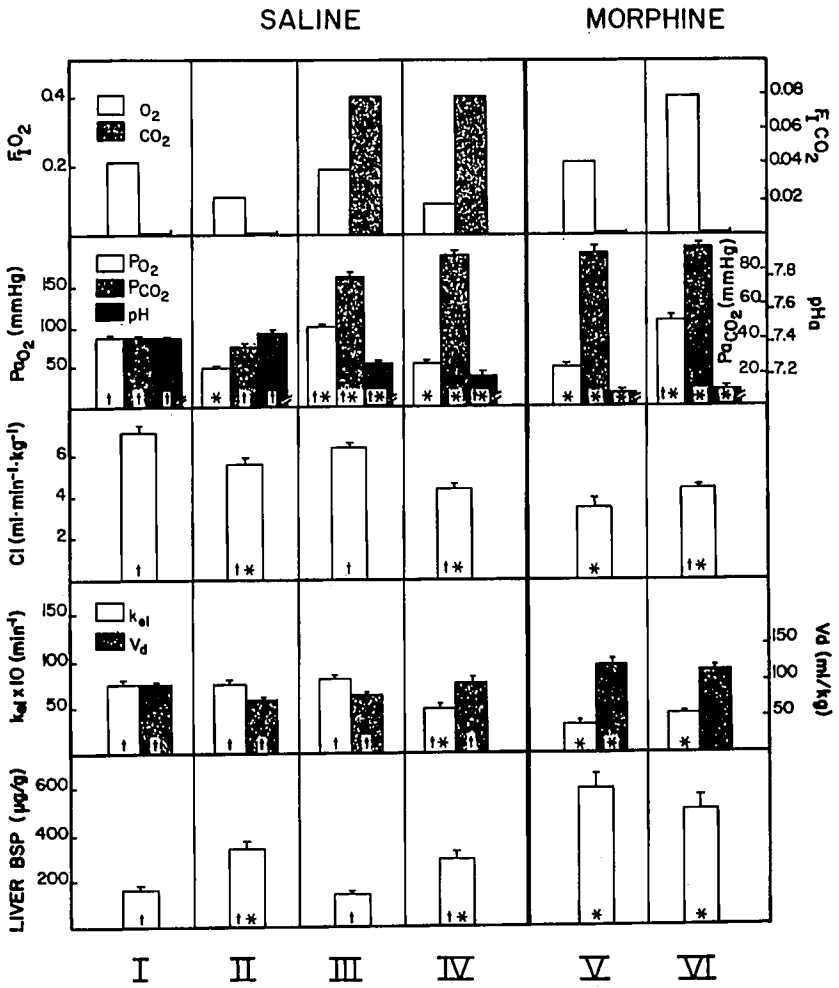

FIG. 1. Effects of blood gas modification and morphine administration on BSP disposition. Rats were exposed to the indicated gas mixtures and given subcutaneous saline or morphine sulfate, 20 mg·kg^{-1}. BSP, 100 mg·kg^{-1}, was given intraarterially. Resultant blood gases, plasma BSP kinetics and liver BSP levels are shown. Data are means ± SEM. N = 8–13 in each group. *$P < 0.05$ compared with saline–air control group. †$P < 0.05$ compared with morphine–air group.

perpnea in these rats kept Pa_{O_2} high, despite slightly decreased FI_{O_2}. Group IV (12 rats) were both hypoxic and hypercarbic. These breathed 9% O_2 and 8% CO_2 to achieve the desired arterial blood gases. In 13 morphine-treated rats, hypoxia was reversed by making them breathe 40% O_2 (Group VI). These rats remained hypercarbic and acidotic.

Sulfobromophthalein sodium (BSP) was obtained from Hynson, Westcott and Dunning, Baltimore, Maryland. Morphine sulfate was obtained from Eli Lilly and Co., Indianapolis, Indiana. Plasma BSP content was determined in a Gilford model 300-N spectrophotometer at 580 nm after appropriate dilution with 0.1 N sodium hydroxide. Liver samples were homogenized in methanol and BSP was determined by the method of Whelan and Combes.[2] Pharmacokinetic parameters of plasma BSP disappearance were calculated by the ESTRIP program of Brown and Manno,[3] according to a two-compartment open model. Clearance (Cl) and volume of distribution (V_d) were calculated from the plasma concentration–time curve ($AUC_{0-\infty}$) for each animal, thus:

$$Cl = \frac{Dose}{AUC_{0-\infty}} \qquad V_d = \frac{Dose}{AUC_{0-\infty} \times k_{el}}$$

Blood gas and *p*H determinations were performed on an Instrumentation Laboratory Model 113 Blood Gas Analyzer.® Data were analyzed for statistically significant differences by analysis of variance and Duncan's test.

Results

The data from all six treatment groups are summarized in figure 1. When given to rats breathing room air (21% O_2, 0.4% CO_2), morphine produced the expected hypoxia, hypercapnia and respiratory acidosis. These morphine-treated rats (Group V) also had much greater plasma levels of BSP as reflected by the halving of its clearance from 7.1 to 3.5 ml min^{-1}·kg^{-1}. The hepatic dye content at 40 min was tripled by morphine, while plasma BSP levels were raised by fivefold as shown in figure 2 ($P < 0.001$). Rats placed in compartments flushed with 11% oxygen became hypoxic to the same extent as caused by morphine (Group II). These animals, which

Downloaded from http://asa2.silverchair.com/anesthesiology/article-pdf/60/6/537/308628/0000542-198406000-00003.pdf by guest on 17 April 2024

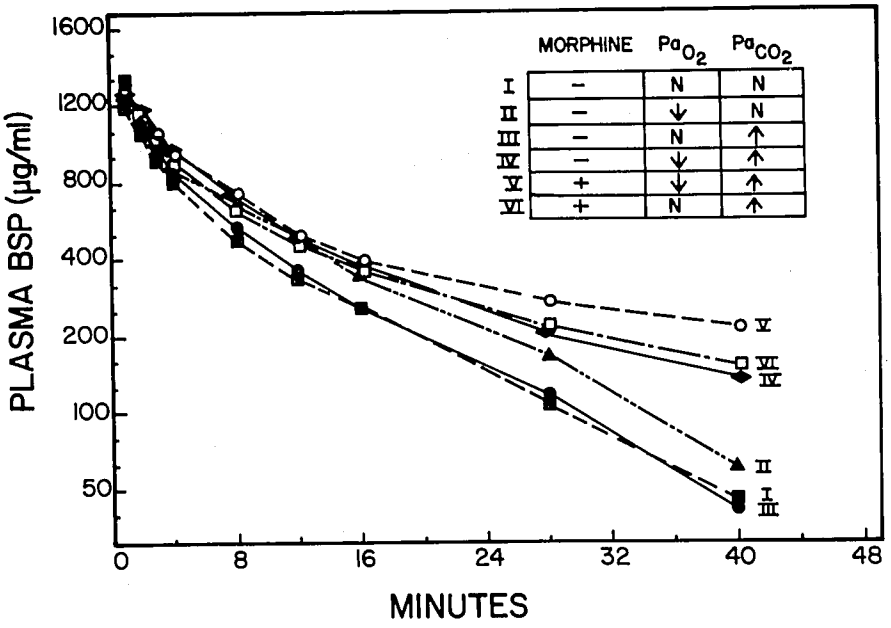

FIG. 2. Effects of blood gas modification and morphine administration on plasma levels of BSP. Blood gas abnormalities were caused, as indicated, by breathing gas mixtures or as a result of morphine-induced respiratory depression. BSP, 100 mg·kg^{-1}, was given intraarterially.

were not hypercapnic or acidotic, did show decreased plasma BSP clearance and had increased hepatic and plasma dye retention—but not to the same extent as caused by morphine. Rats placed in an atmosphere of 19% oxygen and 8% carbon dioxide became hypercapnic and acidotic but did not have lower blood oxygen (Group III). These animals cleared BSP from plasma and liver no differently from saline-treated rats breathing room air. Another group of saline-treated rats were made hypoxic and hypercapnic by being placed in 9% oxygen and 8% carbon dioxide (Group IV). The blood gas pattern in these rats matched that caused by giving morphine to rats breathing room air. In the absence of morphine, hypoxia plus hypercapnia and acidosis did impair removal of BSP from plasma and liver. However, despite identical oxygen and carbon dioxide levels, plasma BSP clearance was significantly less after morphine, while hepatic dye concentrations were twice as high in narcotic-treated animals. Since elevated carbon dioxide and acidosis did not affect BSP disposition, hypoxia appeared to be the common factor in all groups with high plasma and liver dye levels. Morphine-treated rats therefore were placed in compartments flushed with 40% oxygen (Group VI). As expected, respiratory depression caused hypercapnia and acidosis in these animals, but hypoxia was completely reversed. Despite normal blood oxygen levels, morphine treatment decreased plasma BSP clearance by nearly 40% and tripled hepatic dye levels.

Discussion

High-dose narcotics are used in anesthesia[4] and are believed free of serious adverse circulatory effects.[5] We have recently shown that morphine decreased biliary elimination of the anionic dye, sulfobromophthalein (BSP), and increased its levels in plasma and liver.[1] Since narcotics also depress respiration, the present study was designed to explore the relationship between this effect and BSP disposition. Intravascularly administered BSP is taken up by the liver, conjugated with glutathione, and actively secreted into bile. Dye clearance is limited by liver blood flow and is decreased by hepatocellular damage and biliary obstruction. Both hypoxia and hypercapnia have been shown to decrease splanchnic and hepatic blood flow,[6-8] while hypoxia has been shown to suppress hepatic drug metabolism[9] and cause liver damage.[10] The present study shows that hypoxia, but not hypercapnia, decreased plasma BSP clearance and increased hepatic levels of this dye. However, it is unlikely that morphine modifies BSP clearance exclusively by altering blood gases, since the effects of the narcotic were greater than caused by blood gas modification alone. Furthermore, BSP disposition remained normal in hypercarbic and acidotic rats, while plasma and liver dye levels were increased in morphine-treated animals with normal blood oxygen.

To isolate the role of morphine, its effect on BSP disposition in rats with normal blood gases ideally should have been studied. We have found that tight body restraint or general anesthesia (urethane) markedly increase plasma and liver retention of BSP. Such techniques would be needed if rats were to be ventilated through tracheostomies. We chose not to introduce these potentially confounding additional variables and, instead, let the saline and morphine-treated rats move freely in gas-flushed plastic restraining cages. Since we could not control respiration, we were unable to reverse morphine-induced hypercarbia and acidosis. Based on our studies, in which

Downloaded from http://asa2.silverchair.com/anesthesiology/article-pdf/60/6/537/308628/0000542-198406000-00003.pdf by guest on 17 April 2024

morphine-treated rats with normal blood gases were not evaluated, we can suggest, but not conclusively prove, that morphine effects on BSP disposition were not mediated entirely by respiratory depression.

Our earlier study showed that morphine increased liver levels of BSP and decreased its elimination into cannulas placed in the common bile ducts of rats.[1] On the basis of this finding, mechanisms other than biliary spasm were sought to account for the effects of morphine on BSP disposition. In that study, acute biliary cannulation obscured the narcotic's effect on plasma BSP clearance, as may have been expected from the marked decrease in splanchnic blood flow secondary to the abdominal surgery.[11] In the present study, such surgery was avoided to enable evaluation of blood gas changes on BSP levels in plasma as well as liver.

The doses of morphine and BSP in the present study were selected on the basis of our earlier dose ranging experiments.[1] The present morphine dose, 20 $mg \cdot kg^{-1}$, sc, is less than one-twentieth the reported LD_{50} in the rat.[12] It causes behavioral changes and analgesia but the animals remain awake and quite responsive and move about. For small animals, body surface area has been claimed to be more appropriate than body weight as a determinant of drug dosage. If calculated according to surface area,[13] the morphine dose in the present study approximates the 3 $mg \cdot kg^{-1}$ dose used in humans for anesthesia.[5]

We have also found that morphine, fentanyl, and other narcotics increase levels of drugs whose elimination is limited by hepatic blood flow (unpublished results) or by renal clearance.[14] These narcotic effects were reversed by naloxone, suggesting mediation by opioid receptors rather than by competition for excretion pathways. We suggest that care be used in the administration of other drugs together with narcotics, since opioids may potentiate the pharmacologic and toxic effects of other drugs, even if blood oxygen is maintained at a normal level.

References

1. Hurwitz A, Fischer HR: Narcotic effects on hepatic disposition of sulfobromophthalein in rats. J Pharmacol Exp Ther 227:68–72, 1983
2. Whelan G, Combes B: Competition by unconjugated and conjugated sulfobromophthalein (BSP) for transport into bile. Evidence for a single excretory system. J Lab Clin Med 78:230–244, 1971
3. Brown RD, Manno JE: ESTRIP, a BASIC computer program for obtaining initial polyexponential parameter estimates. J Pharm Sci 67:1687–1691, 1978
4. Stanley TH: Narcotics as complete anesthetics, Trends in Intravenous Anesthesia. Edited by Aldrete JA, Stanley TH. Chicago, Year Book, 1980, pp 367–384
5. Lowenstein E, Hallowell P, Levine FH, Daggett WM, Austen WG, Laver MB: Cardiovascular response to large doses of intravenous morphine in man. N Engl J Med 281:1389–1393, 1969
6. Roth A Jr, Rubin RJ: Role of blood flow in carbon monoxide- and hypoxic hypoxia-induced alterations in hexobarbital metabolism in rats. Drug Metab Dispos 4:460–467, 1976
7. Ross WT Jr, Daggy BP: Hepatic blood flow in phenobarbital-pretreated rats during halothane anesthesia and hypoxia. Anesth Analg 60:306–309, 1981
8. Epstein RM, Wheeler HO, Frumin MJ, Habif DV, Papper EM, Bradley SE: The effect of hypercapnia on estimated hepatic blood flow, circulating splanchnic blood volume, and hepatic sulfobromophthalein clearance during general anesthesia in man. J Clin Invest 40:592–598, 1961
9. Jones DP: Hypoxia and drug metabolism. Biochem Pharmacol 30:1019–1023, 1981
10. Shingu K, Eger EI II, Johnson BH: Hypoxia per se can produce hepatic damage without death in rats. Anesth Analg 61:820–823, 1981
11. Gelman SI: Disturbances in hepatic blood flow during anesthesia and surgery. Arch Surg 111:881–883, 1976
12. Barnes CD, Eltherington LG: Drug Dosage in Laboratory Animals, Revised and enlarged ed. Berkeley, University of California Press, 1973, p 165
13. Klaassen CD, Doull J, Evaluation of safety: Toxicologic evaluation, Toxicology: The Basic Science of Poisons, Second edition. Edited by Doull J, Klaassen CD, Amdur MO. New York, Macmillan, 1980, pp 21–22
14. Hurwitz A: Narcotic effects on phenol red disposition in mice. J Pharmacol Exp Ther 216:90–94, 1981

Downloaded from http://asa2.silverchair.com/anesthesiology/article-pdf/60/6/537/308628/0000542-198406000-00003.pdf by guest on 17 April 2024