The Pharmacology of Furosemide in the Horse I. Effects on the Disposition of Procaine, Methylphenidate, Phenylbutazone, and Pentazocine

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Publication #22 from the Kentucky Equine Drug Research Program, Department of Veterinary Science, College of Agriculture, University of Kentucky, Lexington, Kentucky 40506. Published as Kentucky Agricultural Experiment Station article #77-4-126 with the permission of the Dean and Director, College of Agriculture. Supported by grants from the Kentucky Equine Research Fund and the Patricia Hewitt Foundation.

The effects of furosemide (1 mg/kg IV) on the disposition of procaine, methylphenidate, phenylbutazone, and pentazocine in Thoroughbred horses were investigated. Furosemide did not reduce plasma levels of procaine, methylphenidate, or phenylbutazone and had little effect on urinary concentrations of procaine and methylphenidate. Furosemide reduced urinary concentrations of phenylbutazone and the major glucuronide metabolite of pentazocine about 40 to 50-fold.

These results and experience in our laboratory show that furosemide may substantially reduce the probability of detection of phenylbutazone and pentazocine in urine. For phenylbutazone, this problem may be approached either by determining plasma levels of the drug or by using more sensitive detection methods in urine. For pentazocine and other drugs excreted as glucuronides, the solution is not clear-cut. Because of limitations in current analytical technology, it appears likely that furosemide treatment may substantially reduce the probability of detection of many drugs excreted as water soluble metabolites.

Introduction

The extensive use of furosemide[a] in horses racing in some jurisdictions raises questions as to its effect on other drugs with which it may be administered. Thus furosemide might affect the pharmacological response to, disposition of, or urinary elimination of other drugs.[1] Further, and of particular interest to horsemen and racing authorities, furosemide treatment may affect routine forensic screening for other drugs.[1] This study was designed to provide answers to some of these questions.

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[a] Lasix, Hoechst-Roussel Pharmaceuticals, Somerville, NJ

The most likely mechanism for an effect of furosemide on drug disposition is via its renal effects. In the kidney, furosemide enters urine primarily via the tubular organic acid transport system.[6] In the diluting segment of the loop of Henle, it blocks active reabsorption of chloride and thus indirectly that of sodium and water.[7] Furosemide might therefore theoretically affect urinary concentrations of drugs by (a) competing with the tubular secretion of acidic drugs or (b) blocking the renal concentrating mechanism and preventing any concentration of drugs in urine.[2]

Other possible sites for drug interaction also exist. Furosemide might displace other acidic drugs from their plasma protein binding sites and thereby affect their disposition and pharmacokinetics.[2] Furosemide might also affect the rate of metabolism of other drugs and thus their disposition.[12] Conversely, since at least some of the effects of furosemide are mediated via the prostaglandins, drugs such as phenylbutazone might directly antagonize some of the actions of furosemide.[1] To determine the practical significance of these various theoretical possibilities, we studied the effects of furosemide on the disposition of phenylbutazone,[b] procaine,[c] methylphenidate,[d] and pentazocine[e] in horses. Preliminary reports have been communicated.[11,13]

Materials and Methods

(a) Animals—Care and maintenance of horses and collection of urine samples was as previously described.[11] Unless otherwise noted, all experimental points are the means of experiments on at least 4 horses.

(b) Phenylbutazone and Procaine—Plasma and urinary concentrations of phenylbutazone and procaine were determined as previously described.[14,15]

(c) Methylphenidate—Methylphenidate levels in biological fluids were determined by the method of Tobin.[11] One milliliter of saturated sodium tetraborate and 2 ml of cyclohexane were added to 4 ml of the biological fluid or appropriate aqueous standards. The mixture was then "rotoracked" for 5 minutes and centrifuged. Occasionally the "rotoracking" produced a partial emulsion which was resolved by centrifuging at 2,000 x g for 20 minutes. After centrifugation the cyclohexane layer was transferred to another tube and 50 µl of HFBA added. This mixture was allowed to react at room temperature for 3 minutes. At the end of this period, 5 ml of 0.5 M NaOH was added to the mixture and it was again "rotoracked" for 10 minutes. After centrifugation the cyclohexane layer was transferred to a clean tube and an aliquot taken for gas chromatography. Chromatography was on a 3% OV-101 column at an injection temperature of about 300° C. Under these conditions, the retention time for derivatized methylphenidate on the column averaged about 3 minutes.

(d) Pentazocine Glucuronides—Hydrolysis of pentazocine glucuronides was performed by adding to 0.5 ml of urine 1.0 ml of saturated KH_2PO_4, to bring the pH to about 5.0, and 0.8 ml of bovine liver glucuronidase.[f] These were then incubated overnight at 37° C (about 16 hours). Hydrolysis with this relatively large amount of enzyme for this time was required for complete release of all glucuronidase releasable pentazocine.

At the end of the incubation period, 3–4 drops of concentrated NH_4OH were added to bring the pH of the system to about 9.0. Then, 4 ml of dichloromethane was added, the whole rotoracked for 5 minutes, and centrifuged at 1,000 x g for 5 minutes. The dichloromethane phase was then separated and evaporated at 55° C in a water bath to dryness. 0.5 ml of benzene added, and an aliquot of this sample taken for gas chromatography.

Chromatography was on a Perkin-Elmer 3920A equipped with a 3-foot OV-101 column and a nitrogen phosphorous detector. Injection temperature was 210° C, column temperature 220° C, and manifold temperature 250° C. Gas flows were 1 ml/minute H_2 at 8 psi, 100 ml/minute air at 30 psi. The pentazocine peak recovered from equine urine had the same retention time as authentic pentazocine over 3 different column temperatures. Derivatization of the material recovered from equine urine with pentafluoropropionic acid (PFPA) also produced material which chromatographed with similar retention times as PFPA-treated pentazocine.

(e) Drugs, Chemicals and Reagents—Pentazocine was the commercially available injectable form. All other drugs and reagents were as previously described.[12,16]

Results

Although phenylbutazone is known to be highly protein bound in many species, there are no reports on its plasma protein binding in the horse. Figure 1 shows that over the range of drug concentrations found in equine plasma,[17] phenylbutazone is about 95% protein bound. Since furosemide is also acidic and highly protein bound, phenylbutazone and furosemide may share binding sites and thus are candidates for mutual displacement interactions.

[b] "Butazolidin", Jen-Sal Laboratories, Kansas City, MO.

[c] "Novocain", Winthrop Laboratories, New York, NY.

[d] "Ritalin", CIBA Pharmaceuticals, Summit, NJ.

[e] "Lidem", Winthrop Laboratories, New York, NY. Winthrop Laboratories kindly supplied pentazocine standards.

[f] "Glucurase", Sigma Chemical Co., St. Louis, MO.

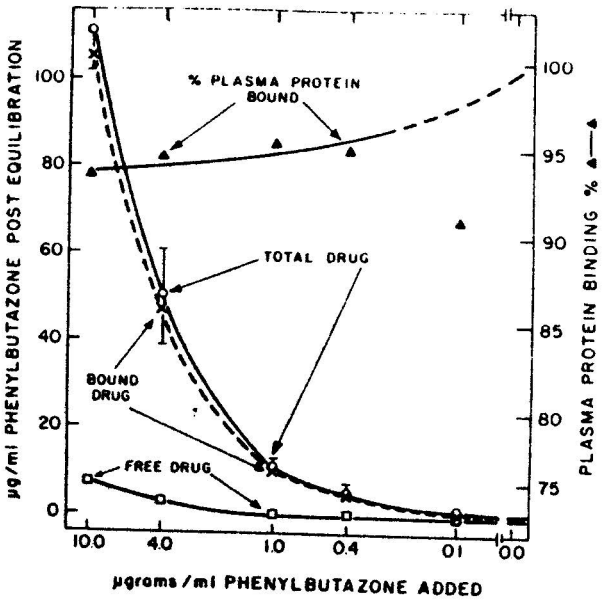

Fig 1—Plasma protein binding of phenylbutazone. The indicated concentrations of phenylbutazone were added to 5 ml of freshly drawn equine plasma and 200 ml of 50 mM phosphate buffer, pH 7.4. The plasma was then placed in a spectrophor membrane dialysis bag, suspended in the phosphate buffer, and the system incubated at 37° C for 18 hours. At the end of this period, the concentrations of phenylbutazone in both phases were determined as described in "Methods." The open squares (□ - □) show the concentration of phenylbutazone remaining in the buffer phase, while the open circles (○ - ○) show concentrations in plasma. The crosses (X - X) represent the difference between these values, which is the concentration of drug plasma protein bound at each point. The solid triangles (▲ - ▲) represent the percent of drug in the plasma protein bound. All experimental points are the means, ± standard error of the mean, of experiments on 3 different equine plasmas.

Figures 2, 3, and 4 show the diuretic effect of 1 mg/kg furosemide IV in these horses and also its effect on the disposition of phenylbutazone in the horse. Phenylbutazone, 6.6 mg/kg, was administered IV at indicated zero times to all animals. The test animals were challenged with 1 mg/kg furosemide IV 2.5 hours after administration of phenylbutazone. Figure 2 shows plasma concentrations of phenylbutazone in these horses. The solid lines show matched experiments in which the same horses were used in both experiments. The crosses represent additional comparable control data from other experiments.[6] Plasma levels of phenylbutazone declined with approximately the same 7-hour half-life in the presence or absence of furosemide, suggesting no clinically significant effect of furosemide on the plasma half-life of phenylbutazone in the horse.

In contrast to the lack of effect on plasma concentrations, urinary concentrations of phenylbutazone were markedly decreased after administration of furosemide (Fig 3). Urinary levels dropped 40-fold within 15 minutes of administration of furosemide and remained depressed for up to 12 hours. Again, the dotted line in Fig 3 is a second control for normal urinary excretion of phenylbutazone taken from other data;[6] the solid line represents a matched control experiment.

Figure 4 shows the effect of furosemide on the rate of urinary excretion of phenylbutazone. As well as reducing the concentration of phenylbutazone in urine, furosemide also tended to reduce the rate of elimination of phenylbutazone since its rate of elimination declined to a low point about 2.5 hours after administration of furosemide. Thereafter, urinary output of phenylbutazone increased, in contrast with the consistent decline observed in controls.

Figure 5 shows the effect of furosemide on plasma and urinary concentrations of procaine. Horses were administered procaine HCl intramuscularly (IM) (10 mg/kg) at indicated zero time, and the test animals were challenged with 1 mg/kg of furosemide IV at 2 hours postprocaine. The open symbols show plasma and urinary levels in the absence of furosemide. The solid symbols show the values in furosemide-treated horses. No statistically significant differences between either group were observed.

Figure 6 shows the rate of excretion of procaine with and without furosemide. Procaine excretion rates after furosemide treatment transiently rose to 20-fold or more than control values, then returned to control values by 4 hours after furosemide treatment. Procaine is lipid soluble at physiological pH and readily distributes throughout the body.[16] As urine volume increased after administration of furosemide, procaine apparently readily entered this expanding compartment and was excreted at a rate proportional to urine flow. Thus furosemide considerably enhanced the urinary excretion rate of procaine.

The effect of furosemide on the disposition of methylphenidate is shown in Fig 7. Plasma levels of methylphenidate in control horses declined with a half-life of about 1 hour after subcutaneous injection of 0.33 mg/kg methylphenidate. Administration of 1.0 mg/kg of furosemide IV 2 hours after the methylphenidate reduced the plasma half-life of methylphenidate while also decreasing the concentration of the drug in urine (Fig 8). The change in urinary concentration observed after furosemide, however, was relatively small, and urinary output of methylphenidate was therefore increased more than tenfold after administration of furosemide (Fig 9).

Figure 10 shows the effects of furosemide on urinary concentrations of a glucuronide metabolite of pentazocine. At indicated zero time, 0.33 mg/kg pentazocine was administered IV to all horses, and the test horses were challenged with 1 mg/kg furosemide IV 30 minutes later. In the absence of furosemide, urinary concentrations of pentazocine fell slowly, from about 100 µg/ml at 30 minutes postdosing to 25 µg/ml at 4 hours. In the presence of furosemide, however, urinary concentrations of the glucuronide metabolite fell sharply, from 100 µg/ml at 30 minutes

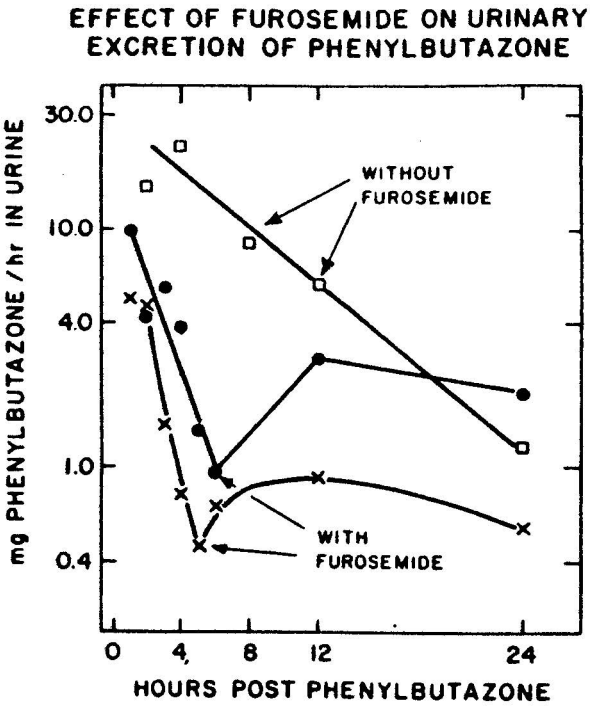

Fig 4—Effect of furosemide on rate of urinary excretion of phenylbutazone. The open squares (□ - □) show the rate of urinary excretion of phenylbutazone after 6.6 mg/kg phenylbutazone IV at zero time. The solid circles (● - ●) and crosses (X -X) show the excretion rates when this dose of phenylbutazone was followed by 1 mg/kg furosemide IV at 2.5 hours. All experimental points are the means of experiments on 4 different horses with standard error of the means omitted for clarity.

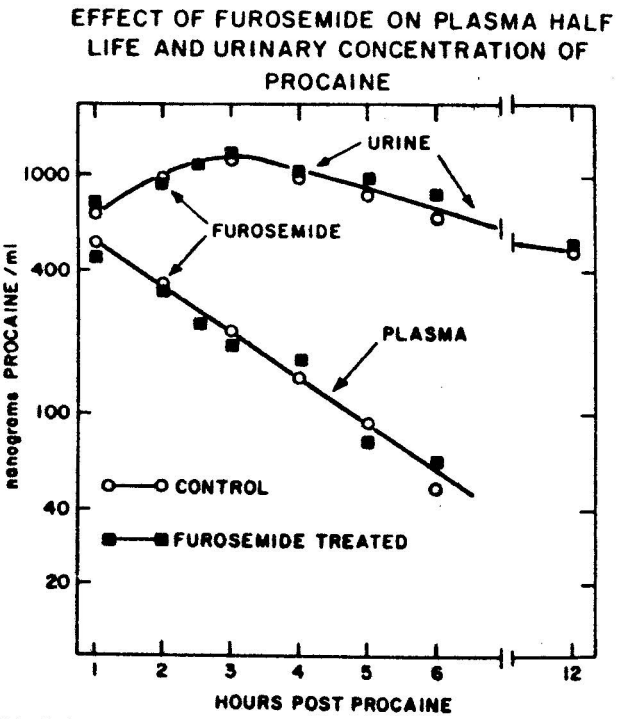

Fig 5—Effect of furosemide on the disposition of procaine in the horse. The open symbols show plasma and urinary concentrations of procaine after intramuscular administration of 10 mg/kg procaine HCl at zero time. The solid symbols show plasma and urinary levels when the administration of procaine was followed by 1 mg/kg of furosemide intravenously. All experimental points are the means of experiments on 4 different horses with standard error of the means omitted for clarity.

had no effect on urinary concentrations of procaine and a relatively small effect (~40% reduction) on urinary concentration of methylphenidate. These observations are in good agreement with those of Frey *et al.*[1] who found little effect of bumetanide treatment on urinary levels of amphetamine and a marked increase in urinary output of this drug during diuresis.

Procaine, amphetamine, and methylphenidate are all basic, relatively lipid soluble drugs, and it seems likely that this accounts for the lack of effect of furosemide on their urinary concentrations. Presumably these drugs can readily diffuse across the renal tubule and equilibrate with urine at concentrations dependent on their pH and the pH of urine. In agreement with this hypothesis, Evans and Lambert[3] have shown that urinary concentrations of procaine are closely related to urinary pH changes, consistent with a rapid equilibration of procaine across renal tubules. Thus, it may be reasonable at this point to assume that furosemide will tend to have little effect on the concentration of basic, highly lipid soluble drugs in equine urine.

In contrast to our observations with basic drugs, urinary concentrations of phenylbutazone were markedly reduced by treatment with furosemide. This effect can be of considerable forensic importance. Our attention was first

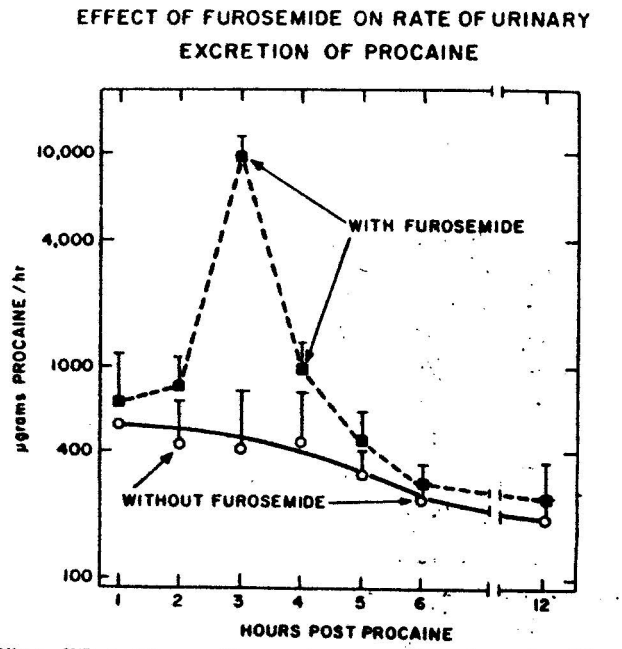

Fig 6—Effect of furosemide on urinary excretion of procaine. The open circles (○ - ○) show urinary elimination of procaine after 10 mg/kg of procaine HCl IM, the solid squares (■ - ■) show elimination of procaine after 1 mg/kg furosemide IV at 2 hours. All experimental points are the means of determinations of 4 different horses ± standard error of the mean.

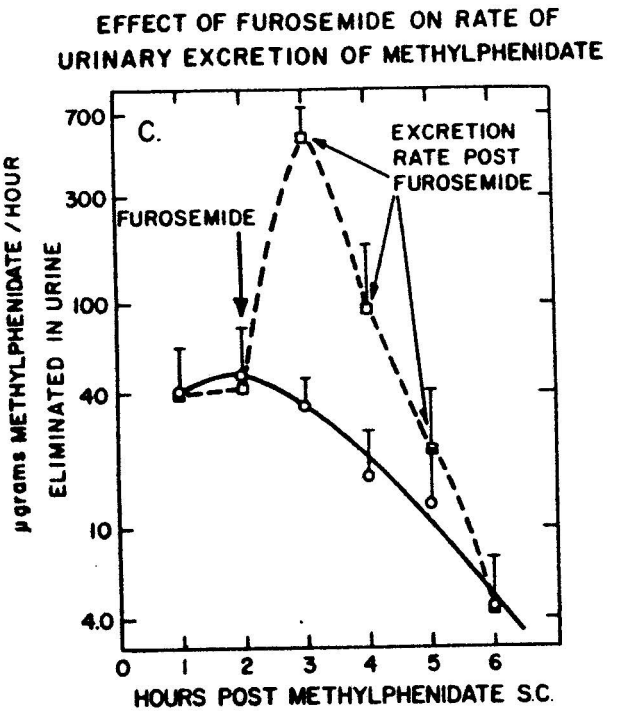

Fig 9—Effect of furosemide on rate of urinary excretion of methylphenidate. The open circles (○ - ○) show the rate of urinary excretion of methylphenidate (0.33 mg/kg) alone, or after 1 mg/kg furosemide IV (open squares, □ - □) 2 hours after administration of furosemide.

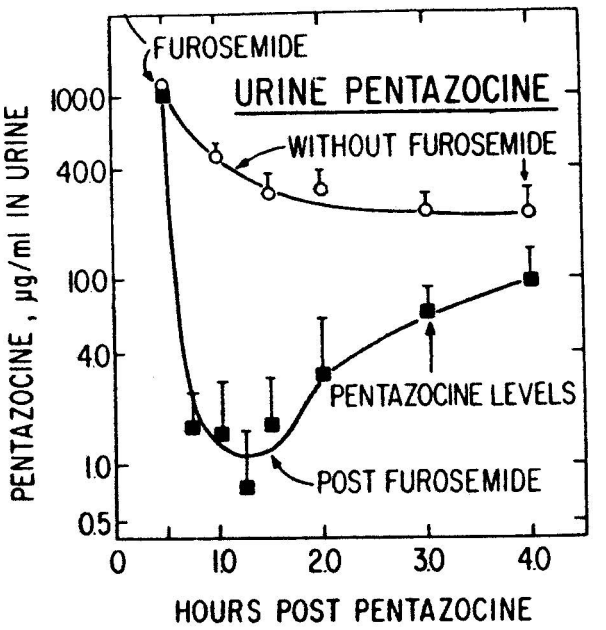

Fig 10—Effect of furosemide on urinary concentrations of a glucuronide metabolite of pentazocine. Horses were injected IV with 0.33 mg/kg pentazocine at indicated zero time. The open circles (○ - ○) show urinary concentrations of a glucuronide metabolite or pentazocine in control horses. The solid squares (■ - ■) show urinary concentrations of this metabolite in horses treated with 1 mg/kg of furosemide IV 30 minutes postpentazocine. All data points are means ± standard error of mean of experiments on at least 4 different horses.

by glomerular filtration or by the organic acid transport system. Since these metabolites are poorly lipid soluble, they are concentrated in urine by the renal concentrating mechanism. Urinary concentrations of these drugs may thus be many times greater than plasma levels of these drugs. Further, because sensitive analytical methods are not available for many drugs excreted as glucuronides, this concentrating effect is important in that it enables analysts to identify many of these drugs during routine forensic screening.*

Pentazocine is rapidly and apparently completely metabolized to a glucuronide metabolite in the horse. Figure 10 shows the effect of furosemide treatment on urinary levels of this metabolite. Urinary concentrations are markedly reduced, by about 50-fold, at peak effect in drug treated animals. As with phenylbutazone, drug levels recovered slowly and were about 50% of control levels at 4 hours postdosing. The experiment shows marked effects of furosemide treatment on urinary levels of a glucuronide metabolite in the horse.

It appears likely that this diluting effect observed for the glucuronide metabolite of pentazocine will apply to other drugs excreted as glucuronides, such as apomorphine, the phenothiazines, fentanyl, and other narcotic drugs. In good agreement with this probability are some recent experiments by Ozog[10] who reports substantial reductions in concentrations of urinary apomorphine in furosemide-treated horses, supporting the hypothesis that glucuronide metabolites are particularly susceptible to diuretic dilution.[11] Further, because many of these drugs are active at extremely low dose levels (phenothiazines) and because analytical methodology for their detection is either marginal or nonexistent (fentanyl), this effect of furosemide on glucuronide metabolites is likely to be highly significant for routine analytical work. Abolition of the renal concentrating effect by furosemide renders detection of these drugs in urine as technically challenging as is their detection at the low levels observed in plasma.

In some recent experiments Frey *et al.*[1] concluded that the diuretic bumetanide "did not interfere with the detection of doping drugs." The results reported here and experience in our Equine Drug Testing Laboratory suggests that this conclusion is in error.[15] Because sample size is limited in routine dope testing, changes in urinary concentrations of the magnitude reported in this report may be critical for drug testing and can mean the difference between success and failure.

As a practical matter, these results show that administration of furosemide can reduce the concentrations of some drugs or their metabolites in urine from 40 to 50-fold. The practical significance of this effect depends entirely on the techniques and skill of the analyst. In our test-