Reprinted from the AMERICAN JOURNAL OF VETERINARY RESEARCH,
Vol. 37, No. 9, Pages: 1107–1110
© American Veterinary Medical Association, 1976. All Rights Reserved.

Pharmacology of Procaine in the Horse: A Preliminary Report

T. Tobin, DVM, PhD; J. W. Blake, PhD; C. Y. Tai, PhD; S. Arnett

SUMMARY

Rapid intravenous injection of 1 g of procaine hydrochloride in Thoroughbred mares produced variable signs of central nervous system excitation for as long as 4 minutes. Plasma concentrations of procaine were similarly variable and transient, decreasing with a half-life of approximately 25 minutes. In vitro, plasma from freshly collected equine blood hydrolyzed procaine with a half-life of approximately 7.5 minutes. This hydrolysis was apparently due to plasma esterases. Penicillin, when added free or complexed as procaine-penicillin, did not protect procaine against hydrolysis by these plasma esterases at pH 7.4.

Procaine has both local anesthetic and central stimulant actions in horses,[5] and as such, its presence in blood or urine of racehorses is prohibited by most racing authorities. However, reports on the pharmacokinetics, behavioral effects, and dose-response relationships of procaine in horses are infrequent, and such information is needed for accurate assessment of the efficacy or otherwise of procaine as a doping drug in horses. Information about the behavioral effects of procaine in horses seems limited to the report of Meyer-Jones,[5] who described signs of excitement in horses dosed with procaine. Occasionally, authors have reported on the esterase activity of equine serum or plasma, and Kunde and Frey[4] suggested that though procaine "is hydrolyzed relatively slowly by horse serum cholinesterase," blood concentrations are not reached after the subcutaneous injection of procaine, which the authors believe would result in central nervous system (CNS) stimulation or a positive influence on racing performance.

The purpose in the present report is to describe preliminary results of an investigation on the pharmacologic features of procaine in Thoroughbred horses.

Materials and Methods

Mature Thoroughbred mares weighing between 454 and 499 kg each were used. For the experimental period, these mares were housed in individual loose boxes and fed hay and water ad libitum. All drug administration, behavioral observations, and sample collections were performed in the mares' "home" loose boxes.

Procaine HCl, procaine base, procaine-penicillin, and physostigmine (eserine) were commercially obtained.[a] The procaine HCl ran as a single spot on thin layer chromatography in methanol or chloroform: methanol (9:1).[8] Procaine HCl was prepared for injection by dissolving it in approximately 30 ml of double-distilled water and sterilized by drawing it through a 45-μm (APD) grid filter. All intravenous (IV) injections were made, and blood samples were collected by jugular venipuncture. Blood samples for plasma procaine estimation were drawn into 15-ml heparinized tubes[b] to which 1 ml of 50% sodium arsenite[c] and 0.05 ml of 1 m*M* physostigmine had been added. Immediately after withdrawing the blood sample, the tube was inverted 3 times to ensure prompt and adequate mixing of esterase inhibitors with the freshly collected blood. The samples were then placed in crushed ice, cooled to 0 C, and centrifuged at 5,000 × g for 10 minutes. The plasma was then separated from the packed erythrocytes and kept for not more than 2 hours at 0 C before analysis for procaine.

For the in vitro experiments, blood was collected into heparinized tubes,[b] cooled, and centrifuged, and the plasma was separated as before. Freshly prepared plasma (2 ml) was then diluted with an equal volume of 60 m*M* phosphate buffer (pH 7.4) and incubated at 37 C. The experiment was started by the addition of approximately 20 μg of procaine HCl and stopped when required by the addition of 1 ml of equal parts of 50% sodium arsenite and 1 m*M* physostigmine. The samples were then analyzed for procaine.

Procaine concentrations in all samples were determined as described by Tobin et al.[8] This method, a modification of that of Brodie et al,[1] involves the alkaline (pH 9.0) extraction of procaine into benzene and returning it to a 1 *N* HCl solution. The concentration of procaine in the HCl is then determined by diazotizing it with sodium nitrite and following this by the addition of *n*-ethylenediamine to give a bright red dye. Absorbance of the red dye at 550 nm is then measured, and the procaine concentration is calculated by comparison with known standards.

Results

By trial and error, a dose (given IV) of 1 g of procaine HCl was found to produce CNS excitation, yet still permit the safe collection of blood samples with minimal restraint at 5 minutes. Twitching, pacing, deep respiration with rapid "blowing" exhalation, and other signs of CNS excitation commenced approximately 30 seconds after the injection was completed. At approximately 1 minute, the mares paced unsteadily in their loose boxes; this pacing continued for 3 more minutes. By the end of the 4th minute, the mares had quieted, and it was usually possible to approach them and collect a blood sample by the 5th minute. In some instances, the mares had resumed

Received for publication July 14, 1975.

From the Equine Drug Research Program, Department of Veterinary Science, University of Kentucky, Lexington, Ky 40506.

This work was done in connection with a project of the Kentucky Agricultural Experiment Station and is published as paper No. 75-4-113, with the permission of the director of the Station.

Supported by a grant from the Kentucky Equine Research Fund.

The authors thank Dr. G. Somes for statistical assistance.

[a] Sigma Chemical Company, St Louis, Mo.

[b] Vacutainer, Becton-Dickinson and Company, Rutherford, NJ.

[c] K & K Laboratories Plainfield, NJ.

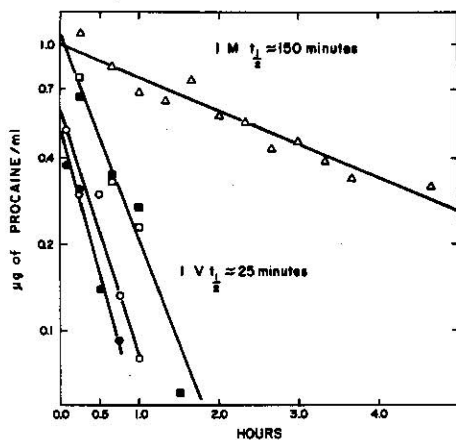

Fig 1—Plasma concentrations of procaine after intravenous (IV) and intramuscular (IM) administration of procaine HCl. △—△ = Plasma concentration of procaine after IM injection of 5 g of procaine HCl in aqueous solution; ●—○ and ■—□ = plasma concentrations of procaine after rapid IV injection of 1 g of procaine HCl in aqueous solution to 4 Thoroughbred mares. All lines were fitted by eye, and the blood concentrations are calculated as procaine base.

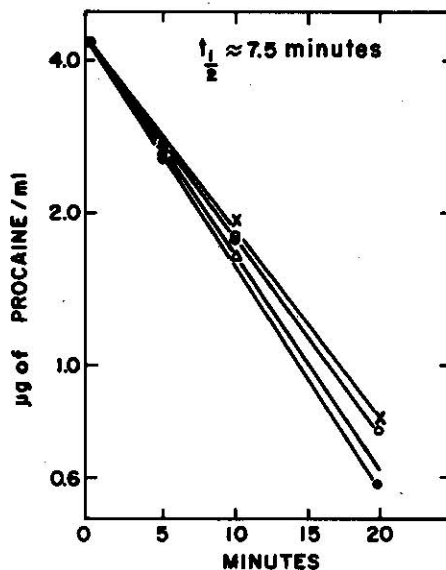

Fig 2—Hydrolysis of procaine by equine plasma in vitro. Freshly collected heparinized blood from Thoroughbred mares was centrifuged and the plasma was separated. To 2 ml of plasma 2 ml of 60 mM phosphate buffer (pH 7.4) and 20 µg of procaine HCl were added. The plasma samples were then incubated at 37 C for the indicated periods, and the reaction was stopped by addition of 1 ml of equal parts of 50% sodium arsenite and 1 mM physostigmine. The plasma samples were then analyzed for procaine as previously described. Each symbol represents plasma samples from different Thoroughbred mares.

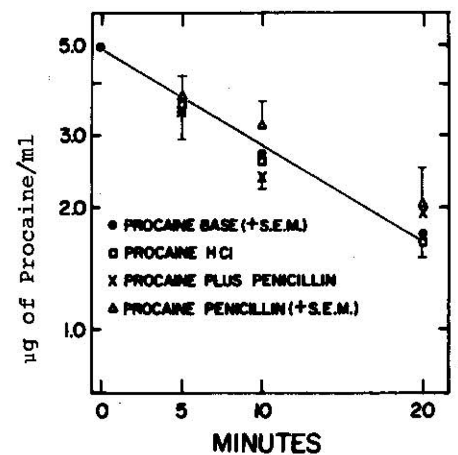

Fig 3—Hydrolysis of procaine-penicillin by equine plasma. The experimental design is as in Figure 2, except that the 20 µg of procaine was added as either procaine base (●), procaine HCl (□), procaine-penicillin (△), or as procaine base plus an equivalent amount of potassium penicillin (×). The procaine HCl was added to the assay system in 20 µl of water; the other substrates were added in 20 µl of isopropanol. Because the stability of the "procaine-penicillin" complex in solution is unknown, all solutions of procaine penicillin were freshly prepared just before their addition to the assay system. All points are the means of experimental determinations on plasma preparations from 4 horses. Standard errors of the means (SEM) are shown for the data points on procaine base and procaine-penicillin. Other SEM are omitted for the sake of clarity. Least square estimates of the slopes for each curve were compared with the slope of the procaine-penicillin curve by means of an F test. None of these slopes are significantly different, with $P > 0.10$ in all cases.

feeding by the 5th minute. However, a 5-minute blood sample was not collected from the 1st pair of horses. In other experiments, the rapid IV injection of equivalent amounts of distilled water or normal saline solution to these mares did not produce observable behavior changes for as long as 2 hours after injection.

Blood concentrations of procaine calculated as micrograms per milliliter of procaine base in 4 Thoroughbred mares given (IV) 1 g of procaine hydrochloride are shown (Fig 1). The blood concentrations observed were variable, but the decrease in plasma concentrations was, in each mare, log linear and with an apparent half-life of approximately 25 minutes. Extrapolating the plasma concentrations back to zero time gave initial blood concentrations between 0.5 and 1.0 µg of procaine/ml. Central nervous system excitation was more marked in the 2 mares which had the higher blood concentrations. The data indicate that, after IV injection of procaine, plasma concentrations rapidly decrease, and CNS excitation due to procaine seems to be associated with plasma procaine concentrations of at least 0.5 µg/ml.

Intramuscular (IM) injection of 5 g of procaine HCl in the most phlegmatic mare in the group caused transient signs of CNS excitation that commenced approximately 5 minutes after injection and lasted approximately 15 minutes (Fig 1). This mare was approached with extreme caution for collecting the 1st blood sample at 20 minutes. Thereafter, however, difficulty was not experienced in collecting blood samples. The data again indicate that blood concentrations in the order of 1 µg/ml are associated with CNS excitation, and that more prolonged blood concentrations of procaine are obtained after IM injection.

Because of the relatively rapid decrease in blood concentrations of procaine after IV injection, the rate of hydrolysis of procaine in equine plasma was investigated. The data presented (Fig 2) indicates that procaine added directly to diluted plasma from 4 different mares disappeared with a half-life of approximately 7.5 minutes.

In other experiments,[9] this hydrolysis did not occur in plasma which had been heated to 100 C for 10 minutes and which had been treated with physostigmine, and significant hydrolysis of procainamide by equine plasma was not observed. Further, significant hydrolysis was not obtained with erythrocytes alone. These observations are consistent, with this hydrolysis being due to one or more plasma esterases.

Since procaine is rapidly hydrolyzed by equine plasma esterases, these esterases presumably contribute, at least in part, to the short plasma half-life of procaine (Fig 1). It has recently been suggested[d] that procaine administered IM as procaine-penicillin may circulate in blood as a procaine-penicillin complex and that the procaine in this complex is protected against esteratic hydrolysis by steric hindrance from the complexed penicillin molecule. A direct test of this hypothesis is shown (Fig 3). In this experiment, the initial rate of hydrolysis

of the procaine-penicillin was not significantly different from that of a system to which procaine and penicillin were added separately. The data indicated that procaine-penicillin added to the esterase system in isopropanol was not protected against hydrolysis by equine plasma esterases. Similar results were obtained in other experiments in which the procaine-penicillin was dissolved in methanol for addition to the incubation system.

Discussion

After IV injection of procaine into horses, plasma procaine concentrations rapidly decreased, with an apparent half-life of approximately 25 minutes. When added to freshly prepared equine plasma, procaine was also rapidly hydrolyzed, disappearing with a half-life of approximately 7.5 minutes. This hydrolytic activity of equine plasma was enzymatic, since it was not observed in heated plasma, and seemed to be due to esterases, since it was blocked by eserine and did not hydrolyze added procainamide.[9] Since the rate of decrease of plasma procaine concentrations in vitro was faster than that in vivo, these plasma esterases presumably make a substantial contribution to the rate of decrease of plasma procaine concentrations in horses in vivo. Other esterases, notably hepatic esterases,[10] must also contribute to the hydrolysis of procaine by horses in vivo, but the quantitative significance of such contribution is not clearly understood.

In other experiments,[9] the initial rate of hydrolysis of procaine by equine plasma decreased in proportion with dilution of the plasma. Thus, the half-life of procaine in undiluted plasma would be approximately 3.7 minutes, or about 6 times the 0.66-minute half-life for procaine in serums from healthy persons as reported by Reidenberg.[7] Similarly, Kisch and Strauss[3] reported that the activity of equine serum esterase is 20 to 12% of that observed in human serums. In agreement with these figures, Kunde and Frey[4] reported that equine serum esterase activity is approximately 16% of that observed in persons. Hazard and Bonomay[2] also showed that blood esterase activity in horses is intermediate between that observed in primates and other domesticated animals. However, a recent (1972) report by Reidenberg[6] indicated that procaine esterase activity is not found in equine serum. The reason for this discrepancy is not clearly understood.

The procaine esterase activity complicates interpretation of the in vivo pharmacokinetic data. Usually, after IV administration of a drug, distinct redistribution and biotransformation phases are identifiable. With procaine, however, biotransformation commences immediately, and a substantial portion of the drug will have been metabolized before redistribution is complete. This rapid metabolism presumably also accounts for part of the variability in the blood concentrations of the drug observed after IV injection. Some of the variability is also accounted for by the small (±5%) variation in the dose of procaine administered when this is calculated on a milligram-per-kilogram basis. However, redistribution to extravascular tissues presumably also occurs, accounting for the longer half-life of procaine in vivo than in vitro.

In the first 2 horses tested, the extrapolated zero time blood concentrations of procaine are approximately 1 μg/ml (Fig 1). Thus, the original 1 g of procaine administered has distributed itself through an apparent volume of distribution of approximately 1,000 L. This large apparent volume of distribution indicates substantial movement of procaine out of the bloodstream to tissue-binding sites. Since the principal sites for metabolism of procaine are probably liver and plasma, such tissue binding of procaine presumably serves to protect it against hydrolysis and prolongs its half-life beyond the approximate 7.5-minute half-life expected in the vascular system. Similarly, IM injection of procaine (Fig 1) prolongs plasma concentrations of the drug by allowing its slow release into the bloodstream.

One important mechanism by which procaine could be protected against hydrolysis by procaine esterase would be by steric hindrance from a complexed molecule. Thus, the salt of procaine-penicillin is poorly soluble in water, and it has been suggested that, after its IM injection, procaine-penicillin is absorbed directly into the bloodstream and circulates as a procaine-penicillin complex.[d] In this way, a proportion of the procaine circulating in horses given procaine-penicillin could be protected against hydrolysis by plasma esterases. Results in the present experiments indicate that procaine added as procaine-penicillin in isopropanol was hydrolyzed at the same rate as free procaine added to the system. These results indicate that either bound penicillin does not sterically hinder the action of the plasma esterases or procaine-penicillin complex dissociates under these experimental conditions so rapidly that the availability of free procaine is not rate limiting for its esteratic hydrolysis.

The observation that procaine added as procaine-penicillin was hydrolyzed by these esterases at the same rate as authentic procaine allows only 2 major interpretations (i.e., the molecules had dissociated to give rise to free procaine or that procaine was split in situ on the complex). Though it seems unlikely that complexed procaine would be hydrolyzed at the same rate as free procaine, data (Fig 3) alone do not allow one to choose between these possibilities. However, results in other experiments[e] on the partition coefficients of procaine and procaine-penicillin from aqueous environments between pH 5.0 and 9.0 into a number of different nonpolar solvents have not shown differences in the partitioning of procaine from either source. These experiments are consistent with rapid and complete dissociation of the procaine-penicillin complex in aqueous environments and thus support suggestions that hydrolysis occurs because procaine is free from the penicillin moiety.

All horses involved in these tests had signs of CNS excitation shortly after the administration of procaine, and this excitation was most marked and prolonged after the IM

[d] Monti, G. W.: Some Aspects of the Metabolism of Procaine Penicillin in the Dog. MSc Thesis, Department of Veterinary Physiology and Pharmacology, the Ohio State University, Columbus, Oh, 1975.

[e] Tobin, T., Blake, J. W., O'Leary, J., Tai, C. Y., Sturma, L., and Arnett, S.: Pharmacology of Procaine in the Horse. III. The Procaine-Penicillin Complex and Its Forensic Significance. Submitted for publication, Am J Vet Res, 1975.

dose of procaine was given. According to results, plasma concentrations of greater than 0.8 μg of procaine/ml are required for procaine to produce signs of CNS excitation. These observations agree well with the data of Kunde and Frey,[4] who reported signs of CNS excitation were not seen in horses which had blood concentrations as great as 0.63 μg of procaine/ml.

References

1. Brodie, B. B., Lief, P. A., and Poet, R.: The Fate of Procaine in Man Following Its Intravenous Administration and Methods for the Estimation of Procaine and Diethylaminoethanol. J Pharmacol Exp Ther, 96, (1948): 359–366.
2. Hazard, R., and Bonomay, C.: Hydrolyse de la procaine (novochine) par le sant de Quelques Animaux. C R Soc Biol (Paris), 142, (1948): 743–745.
3. Kisch, B., and Strauss, Eduard: New Experiments with Procaine Esterase. Exp Med Surg, 1, (1943): 367–370.
4. Kunde, Von M., and Frey, H. H.: Beitrag zur Frage des "doping" mit procain. Berl Munch Tieraerztl Wochenschr, 84, (1971): 14–15.
5. Meyer-Jones, L.: Miscellaneous Observations on the Clinical Effects of Injecting Solutions and Suspensions of Procaine Hydrochloride into Domestic Animals. Vet Med (Praha), 45, (1951): 435–537.
6. Reidenberg, M. M.: The Procaine Esterase Activity of Serum from Different Mammalian Species. Proc Soc Exp Biol Med, 140, (1972): 1059–1061.
7. Reidenberg, M. M.: The Rate of Procaine Hydrolysis in Serum of Normal and Diseased Human Subjects. Clin Pharmacol Ther, 13, (1972): 279–284.
8. Tobin, T., Tai, C. Y., and Arnett, S.: Recovery of Procaine from Biological Fluids. Res Commun Chem Pathol Pharmacol, 11, (1975): 187–194.
9. Tobin, T., Blake, J. W., Tai, C. Y., Sturma, L., and Arnett, S.: Pharmacology of Procaine in the Horse. II. The Procaine Esterase Properties of Equine Plasma and Synovial Fluid. Am J Vet Res, 37, (1976): In press.
10. Yeary, R. A., and Gerken, D. A.: Hepatic Drug Metabolism in Vitro in the Horse. Biochem Pharmacol, 20, (1971): 3209–3210.