Comparison of Effects of Cyanide and Acetylcholine on Renal Hemodynamics and Sodium Excretion

RONALD L. WILLIAMS *[x], JAMES E. PEARSON [‡], and FRANCISCO M. GONZALEZ [‡]

Received March 18, 1981, from the *Department of Pharmacology and the [‡]Department of Medicine, Louisiana State University Medical Center, New Orleans, LA 70112. Accepted for publication May 14, 1981.

Abstract □ The present study compared the effects of acetylcholine to cyanide under the same experimental conditions of renal clearance in anesthetized dogs. Since cyanide is one of the few drugs for which the mechanism of action is known (cytotoxic hypoxia), some insight may be gained into the renal effects of acetylcholine since both produce direct natriuresis and diuresis. Infusion of 0.2 μg/kg/min of acetylcholine and 12.0 μg/kg/min of sodium cyanide into the left renal artery resulted in similar effects, *i.e.*, increased fractional excretion of sodium, potassium, calcium, and magnesium. These effects were immediate and ipsilateral. Both agents increased the renal plasma flow to the same extent. In addition, regression plots of the relation between changes in sodium excretion and changes in renal plasma flow were similar for both agents. The pattern of similar renal functional changes suggested that acetylcholine is not a mere renal vasodilator but that its action is also mediated through alterations on direct tubular transport of ions.

Keyphrases □ Hemodynamics, renal—effects of cyanide and acetylcholine compared □ Cyanide—effects on renal hemodynamics and sodium secretion, comparison with acetylcholine □ Acetylcholine—effects on renal hemodynamics and sodium secretion, comparison with cyanide

May and Carter (1) showed that something other than simple vasodilation is responsible for the saluretic action of cholinergic drugs. When they infused cholinergic drugs into the renal portal system of chickens, there was an ipsilateral saluresis; however, noncholinergic drugs (isoproterenol, papaverine, and dopamine) produced no saluresis despite similar renal vasodilation.

BACKGROUND

The prevalent conclusion has been that vasodilating agents induce changes in sodium excretion mainly by altering renal blood flow (2, 3). Using autoperfused kidneys, no correlation was found between changes in renal perfusion pressure and changes in sodium excretion for either acetylcholine or isoproterenol (4). Itskovitz and Campbell (5) infused seven different vasodilators into isolated blood-perfused canine kidneys at constant pressure. Despite similar hemodynamic effects on renal blood flow, perfusion pressure, and intrarenal distribution of blood flow, their effects on glomerular filtration rate (*GFR*), sodium excretion, and urine flow were dissimilar. Acetylcholine was by far the most natriuretic. Eledoisin, one of the vasodilators examined, actually decreased sodium excretion.

Stein *et al.*, (6) showed that acetylcholine (in micropuncture studies) decreased fractional and absolute sodium reabsorption in the proximal tubule. During this depression of proximal tubular reabsorption of sodium, there was no redistribution of glomerular filtration to outer cortical nephrons. Hayslett *et al.* (7), using a split-droplet method of micropuncture, infused acetylcholine into the abdominal aorta of rats and obtained a natriuresis despite a 50% reduction of glomerular filtration and renal blood flow. However, they interpreted the natriuresis to be due to intrarenal hydrostatic pressure and extraepithelial physical factors.

This study investigated renal vasodilation and the relation between hemodynamic and natriuretic changes by comparing intrarenal infusion of cyanide to that of acetylcholine.

EXPERIMENTAL

Materials and Methods—Five mongrel dogs were anesthetized with pentobarbital sodium, 30 mg/kg iv. Both ureters were cannulated through an abdominal midline incision, and the cannulas were positioned ~2 cm below the ureteral pelvic junction. A femoral vein and artery were cannulated, and the arterial cannula was connected to a transducer with a three-way stopcock for recording blood pressure with a polygraph. Arterial blood samples were obtained through the three-way stopcock.

Solutions containing creatinine, *p*-aminohippurate, and normal saline were infused at a rate of 5 ml/min through the venous system using a dual-syringe constant-flow infusion pump. A 27-gauge hypodermic needle, attached to a number 10 polyethylene tube, was placed in the left renal artery in the direction of blood flow. Through this renal arterial system, a solution of isotonic sodium chloride was continuously infused at a rate of 0.1 ml/min.

Solutions of drugs were also infused through the system at the same rate by changing the renal arterial infusate to one containing test drugs dissolved in normal saline. Both acetylcholine and sodium cyanide were infused into the same animal under the same experimental conditions. One to two hours was allowed for equilibration, and then collections of 10-min urine samples from each kidney were begun. Blood samples, drawn every 20 min from the femoral artery, were heparinized and centrifuged, and the plasma was immediately removed. At least three control urine samples were collected before these agents were infused into the kidney. The order of drug infusion was altered in some experiments.

Analysis—Chloride concentrations in urine and plasma were determined using a titrametric chloridometer; sodium and potassium concentrations in urine and in plasma were determined using a flame photometer with an internal lithium standard; a modification of a previous method (8) was used in the creatinine determinations. *p*-Aminohippurate determinations were made using a reported method (9). Tubular maximal secretion rates of *p*-aminohippurate were obtained by infusing high concentrations intravenously and then subtracting the amount filtered from the total excreted (10). The osmolalities were determined by freezing-point depression.

In addition to the electrolyte excretion rates, the glomerular filtration rates, the effective renal plasma flow, and the tubular rejection fraction of sodium were calculated. Calcium and magnesium were measured by atomic absorption spectrophotometry. Statistical methods for regression lines and significance of mean differences were taken from Snedecor (11).

Table I—Comparison of Intrarenal Infusion of Acetylcholine to that of Sodium Cyanide[a]

Parameter	Acetylcholine, 0.88 nmole/kg/min Δ[b]	*SE*	Sodium Cyanide, 0.25 μmole/kg/min Δ	*SE*
V, ml/min	1.03	±0.22	2.89	±0.72
C_{osm}, ml/min	0.99	±0.24	2.48	±0.57
GFR, ml/min	1.3	±1.8	3.0	±1.0
C_{PAH}, ml/min	11	±5.0	11	±3.8
$U_{Na^+}V$, μEq/min	145	±32	359	±109
$U_K V$, μEq/min	9	±4	28	±18
$U_{Ca^{2+}}V$, μEq/min	14	±6	15	±7
$U_{mg^{2+}}V$, μEq/min	4.0	±2	3.8	±1.2

[a] All drugs were infused into the left renal artery. [b] Each value is the mean change from five dogs obtained by subtracting the changes in the right, control kidneys from changes in the left, infused kidneys; Δ = (left kidney: drug period − control period) − (right kidney: drug period − control).

0022-3549/82/0100-0047$01.00/0

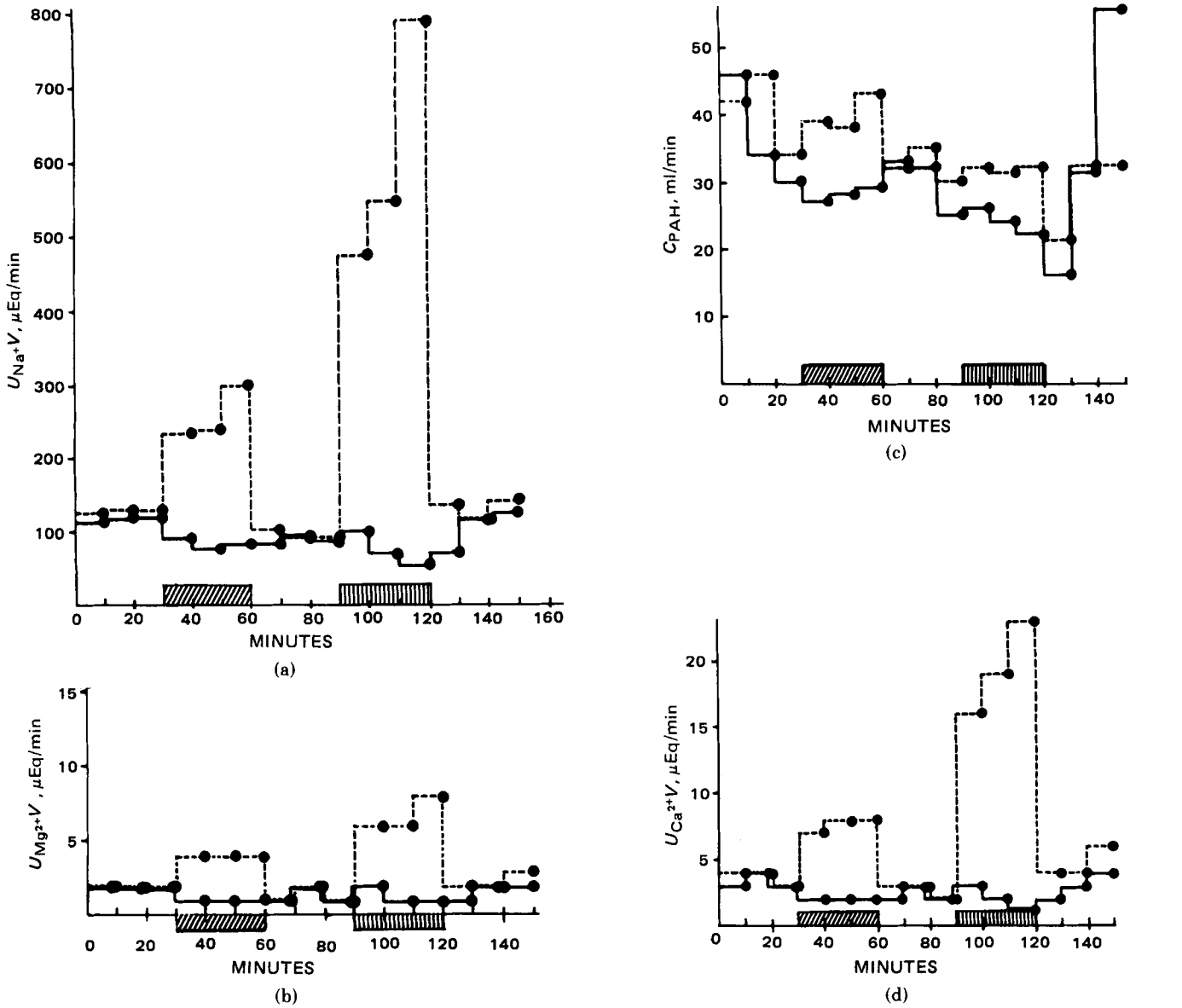

Figure 1—*Comparison of renal effects of cyanide and acetylcholine during infusion into the left renal artery. Key: - - -, left; —, right;* ▨, *acetylcholine bromide (0.2 μg/kg/min); and* ▥, *sodium cyanide (12.0 μg/kg/min).*

RESULTS

The effects of acetylcholine and local hypoxia on renal function were compared during the infusion of 0.2 μg/kg/min of acetylcholine bromide and 12.0 μg/kg/min of sodium cyanide into the left renal artery of the same animal at different times (Figs. 1*a* and 1*b*). (Converted to a molar basis, the infusion rate would be 0.88 nmole of acetylcholine bromide and 0.25 μmole of sodium cyanide/kg/min, respectively.) During the infusion of each drug, there were immediate ipsilateral increases in sodium, potassium, and calcium excretion, in osmolar clearance, creatinine clearance, *p*-aminohippurate (PAH) clearance, and urine flow. When either drug infusion was stopped, there was an immediate return to control values, even for cyanide.

Both acetylcholine and cyanide actually increased *p*-aminohippurate secretion from the tubules (Fig. 2).

The relationship between changes in sodium excretion ($U_{Na^+}V$) and changes in renal plasma flow (*RPF*) are shown in Figs. 3 and 4. The regression line for acetylcholine (Fig. 3) was $U_{Na^+}V = 8.71\ (RPF) + 29$ with a correlation coefficient of 0.83 ($p < 0.05$); the regression line for cyanide (Fig. 4) was $U_{Na^+}V = 11.5\ (RPF) + 44$ with a correlation coefficient of 0.89 ($p < 0.05$). Therefore, with the assumption of a linear relationship, cyanide produced the same effect as acetylcholine on sodium excretion and renal blood flow.

To exclude systemic influences from direct renal effects during acetylcholine or cyanide infusion into the left renal artery, the data were arranged in Table I in the following manner: (*a*) mean changes in the excretion of fluid and electrolytes were calculated along with their respective standard errors; and (*b*) each change was obtained by subtracting the changes in right, noninfused kidneys from the changes in left, experimental kidneys. Both acetylcholine and sodium cyanide produced increased excretion of each electrolyte.

DISCUSSION

Both agents increased renal blood flow and increased tubular secretion of *p*-aminohippurate. The natriuretic effects of cholinergic drugs are of the muscarinic type inhibited by atropine (12, 13). Intrarenal infusion of the muscarinic agent bethanechol (12) produced natriuresis, which was unrelated to washout of the medullary concentration gradient. No correlation was shown (14) between sodium excretion and medullary blood flow when acetylcholine was infused into a renal artery. It was postulated (13) that cholinergic drugs produce saluresis by direct action on the renal tubule, but the influence of vasodilation could not be eliminated. Cyanide, which is known to depress the energy from the electron transport system for active sodium reabsorption, also produces vasodilation. Vasodilation does not explain the natriuresis of cyanide and, therefore, may not explain the natriuresis of acetylcholine. Although a correlation between two variables does not prove a cause and effect relationship, this comparison of acetylcholine with cyanide does add indirect evidence that acetylcholine acts directly on renal tubular sodium transport in some way.

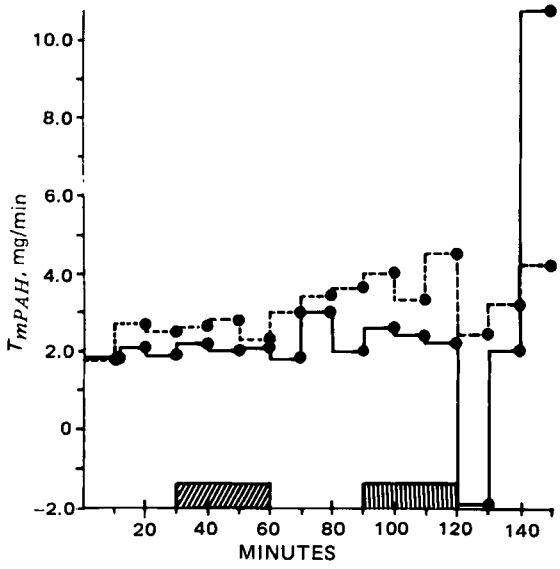

Figure 2—*Effects of intrarenal acetylcholine bromide and cyanide on tubular secretion of* p-*aminohippurate* (T_{mPAH}) *Key: - - -, left; — right;* ▨, *acetylcholine bromide (0.2 μg/kg/min); and* ▥, *sodium cyanide (12.0 μg/kg/min).*

The direct natriuretic effect of cholinergic agents was shown in chicken (12, 15) and dog (13, 16–18) kidneys. Some workers concluded direct tubular effects from micropuncture studies (6, 19); others interpreted the saluretic effect to be a result of intrarenal physical alterations due to hemodynamic changes from vasodilation (2, 3, 7). However, papaverine, a noncholinergic vasodilator, was found to be natriuretic in anesthetized sheep but not in conscious sheep (20). In conscious sheep, there was an inverse relationship between sodium excretion and changes in renal blood flow. In fact, there was a reduction in sodium excretion from the conscious animals despite an increase in renal blood flow. Cholinergic agents were shown (1) to be natriuretic in chickens, but noncholinergic vasodilators were not natriuretic even though blood flow was increased. The natriuretic action of acetylcholine cannot be explained by a redistribution of blood flow to shorter cortical nephrons (6).

The physiological importance of acetylcholine is an interesting question

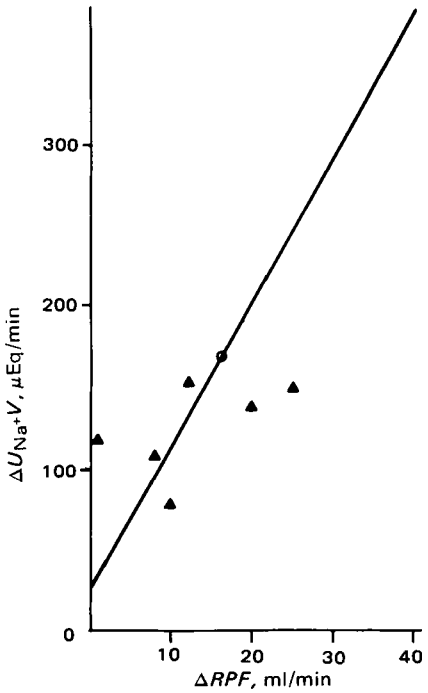

Figure 3—*Linear regression for changes in sodium excretion* ($U_{Na^+}V$) *and changes in renal plasma flow* (RPF) *during infusion of acetylcholine bromide into the left renal artery* ($y = bx + c$, $\Delta U_{Na^+}V = 8.71$ (ΔRPF) + 29, $\bar{y} = 172 \pm 47$, $\bar{x} = 16 \pm 4$, $r = 0.83$, *and* $p < 0.05$).

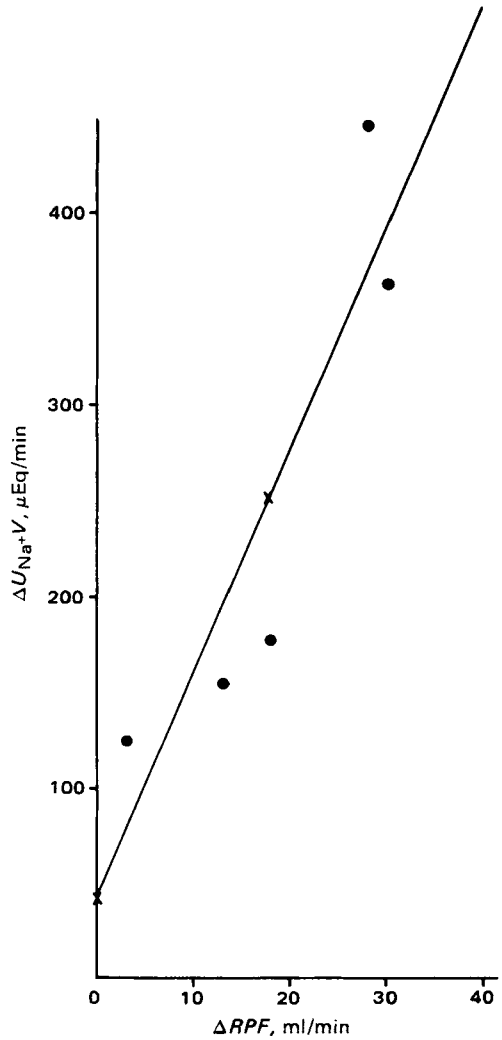

Figure 4—*Linear regression for changes in sodium excretion and changes in renal plasma flow* (RPF) *during infusion of sodium cyanide into the left renal artery* ($y = bx + c$, $\Delta U_{Na^+}U = 11.5$ (ΔRPF) + 44, $\bar{y} = 254 \pm 14$, $\bar{x} = 18 \pm 11$, $r = 0.89$, *and* $p < 0.05$).

since renal tubules were shown (21) to be directly innervated by autonomic nerves. In addition, the renal effects of acetylcholine are similar to cyanide, which inhibits aerobic metabolism equally in the renal cortex and outer medulla (10, 22, 23), resulting in natriuresis. Cyanide-induced natriuresis was one of the first observations relating sodium transport in the kidney to oxidative metabolism (24). Surprisingly, cyanide does not inhibit *p*-aminohippurate secretion (25); therefore, its clearance can be used to measure effective renal plasma flow. Cyanide also increases renal blood flow (23, 26).

In this study, the slopes of the lines for the function $U_{Na^+}V = b(RPF) + c$, where b is the slope of the line and c is the y intercept for a line ($y = bx + c$), for acetylcholine and cyanide were not significantly different (Figs. 3 and 4), assuming linear regression. The pattern of electrolyte excretion was apparently the same for each drug, including that of sodium, potassium, calcium, and magnesium. Just as cyanide acts on both the proximal and more distal portions of the nephron (23), so does acetylcholine according to micropuncture work (6). Therefore, *in vivo* comparison of acetylcholine and cyanide under identical clearance conditions in dogs substantiates that acetylcholine is not merely a vasodilator but either acts directly on tubular transport or increases the permeability and back-leak of sodium, causing a net increase in sodium excretion. There were increases in volume of urine flow, osmolar clearance, and excretion of potassium, calcium, and magnesium. Both acetylcholine and cyanide increased effective renal plasma flow.

REFERENCES

(1) D. G. May and M. K. Carter, *Am. J. Physiol.*, **218**, 417 (1970).
(2) J. A. Martino and L. E. Earley, *Circ. Res.*, **23**, 371 (1968).

(3) L. R. Willis, J. H. Ludens, J. B. Hook, and J. E. Williamson, *Am. J. Physiol.*, **217,** 1 (1969).
(4) R. L. Williams, J. E. Maines, and J. E. Pearson, *Arch. Int. Pharmacodyn. Ther.*, **196,** 393 (1972).
(5) H. D. Itskovitz and W. B. Campbell, *Proc. Soc. Exp. Biol. Med.*, **153,** 161 (1976).
(6) J. H. Stein, J. H. Reineck, R. W. Osgood, and T. F. Ferris, *Am. J. Physiol.*, **220,** 227 (1971).
(7) J. P. Hayslett, D. T. Domoto, M. Kashgarian, and F. H. Epstein, *Am. J. Physiol.*, **218,** 880 (1970).
(8) R. W. Bonsnes and H. H. Taussky, *J. Biol. Chem.*, **158,** 581 (1945).
(9) A. C. Bratton and E. K. Marshall, Jr., *J. Biol. Chem.*, **128,** 537 (1939).
(10) R. L. Weiner and T. W. Skulan, *Am. J. Physiol.*, **221,** 86 (1971).
(11) G. W. Snedecor, "Statistical Methods," 5th ed., Iowa State University, Iowa, 1956.
(12) B. Avrunin and M. K. Carter, *Proc. Soc. Exp. Biol. Chem.*, **133,** 901 (1970).
(13) R. L. Williams, J. E. Pearson, and M. K. Carter, *J. Pharmacol. Exp. Ther.*, **147,** 32 (1965).
(14) K. Aukland and E. W. Loyning, *Acta Physiol. Scand.*, **79,** 95 (1970).
(15) D. G. May and M. K. Carter, *Am. J. Physiol.*, **212,** 1351 (1967).
(16) A. J. Vander, *Am. J. Physiol.*, **206,** 117 (1963).
(17) A. R. Lavender, I. Aho, and T. N. Pullman, *Proc. Soc. Exp. Biol. Med.*, **119,** 887 (1965).
(18) J. E. Pearson and R. L. Williams, *Br. J. Pharmacol.*, **33,** 223 (1968).
(19) G. R. Marchand, C. E. Ott, F. C. Long, R. F. Greger, and F. G. Knox, *Am. J. Physiol.*, **232,** F 147 (1977).
(20) E. H. Blaine, *Proc. Soc. Exp. Biol. Med.*, **158,** 250 (1978).
(21) J. Muller and L. J. Barajas, *Ultrastruct. Res.*, **41,** 533 (1972).
(22) M. Fujimoto, F. D. Nash, and R. H. Kessler, *Am. J. Physiol.*, **260,** 1237 (1964).
(23) N. Martinez-Maldonado, G. Eknoyan, and W. N. Suki, *Am. J. Physiol.*, **217,** (1964).
(24) E. H. Starling and E. B. Verney, *Proc. R. Soc. London Ser. B.*, **97,** 321 (1925).
(25) J.C. Strickler and R. H. Kessler, *Am. J. Physiol.*, **205,** 117 (1963).
(26) J. Johannesen, M. Lie, and F. Kiil, *ibid.*, **233,** F 207 (1977).

ACKNOWLEDGMENTS

Presented in part at the 62nd annual meeting of the Federation of American Societies for Experimental Biology, Atlantic City, N.J., 1978.

Kinetics of Chlorambucil Hydrolysis Using High-Pressure Liquid Chromatography

DULAL C. CHATTERJI ˣ, RUSSELL L. YEAGER, and JOSEPH F. GALLELLI

Received October 23, 1980, from the *Pharmaceutical Development Service, Pharmacy Department, Clinical Center, National Institutes of Health, Bethesda, MD 20205.* Accepted for publication May 12, 1981.

Abstract □ A stability-specific high-pressure liquid chromatographic (HPLC) method was developed to assay intact chlorambucil (I) in the presence of its hydrolytic decomposition products. The HPLC method was used to follow the degradation kinetics of I over pH 1.0–10.0 in the presence of various buffers with and without added chloride ion. In the absence of chloride ion, the hydrolysis of I followed first-order kinetics and the pH rate profile showed a sharp inflection around pH 2.5 attributable to the ionization of the nitrogen mustard and a shallower inflection around pH 5.0 attributable to the ionization of the carboxylic group. The rate was pH independent over pH 6.0–10.0 and independent of buffer species in the absence of chloride ion. In the presence of chloride ion, the kinetics of I hydrolysis was still first order. However, the degradation half-life at a particular pH and buffer concentration increased linearly with chloride concentration. Kinetic evidence is presented to show that the mechanism of chloride stabilization involves the attack of chloride ion on the unstable cyclic ethyleneimmonium intermediate to give back I. Implications of the kinetic data obtained on the fate of orally administered I are discussed.

Keyphrases □ Chlorambucil—kinetics of hydrolysis, high-pressure liquid chromatography □ Hydrolysis—chlorambucil, analysis of kinetics using high-pressure liquid chromatography □ High-pressure liquid chromatography—analysis of chlorambucil hydrolysis kinetics □ Kinetics—chlorambucil hydrolysis, high-pressure liquid chromatography

Nitrogen mustards were one of the first classes of cancer chemotherapeutic agents systematically studied, and they still are used clinically (1). Although the general mechanisms of hydrolysis and alkylation for nitrogen mustards are well known (1–6), little detailed kinetic data are available on their hydrolysis. One reason for the lack of comprehensive kinetic work on the mustards has been the lack of suitable stability-specific analytical methods.

BACKGROUND

Most early work on the kinetics of mustards was performed by estimating the amount of free chloride ion liberated. However, this indirect method of determining intact nitrogen mustards and subsequent kinetic analysis can lead to incorrect stability estimates (3). Furthermore, the stabilizing effect of chloride on mustard hydrolysis cannot be quantitatively evaluated with this method. More recent reports (4, 5) on the hydrolysis of melphalan using stability-specific high-pressure liquid chromatography (HPLC) produced some useful information on the kinetics of melphalen hydrolysis and on the effect of chloride ion on its stability.

Chlorambucil (I), an aromatic nitrogen mustard, is considered stable enough to be administered primarily as an oral dosage form. However, there are no reported data on the stability of I over the GI pH range at 37°. It was recently shown (3), using a stability-specific HPLC method for I, that the half-life of I degradation was only 25 min at pH 3.0 and 37°. Since pH 3.0 is not unrealistic for the stomach, these data showed potential stability problems for orally administered I. Thus, a kinetic study of I hydrolysis over the pH range of the GI system seemed warranted.

EXPERIMENTAL

Materials—Chlorambucil USP reference standard was used for all kinetic studies. Water was double distilled in an all-glass apparatus, and glass-distilled solvents[1] were used for HPLC. All other chemicals were reagent grade.

Buffers—Citrate buffers (pH 2.0–7.0), acetate buffers (pH 3.5–5.5), phosphate buffers (pH 6.0–9.0), and borate buffers (pH 8.5–10.0) were prepared from the 0.25 *M* stock solutions of citric acid, acetic acid, monobasic sodium phosphate, and boric acid, respectively, by the addition of sodium hydroxide solution to the desired pH. Addition of chloride ion was avoided except when specified. Ionic strength was maintained con-

[1] Burdick & Jackson Laboratories, Muskegon, Mich.

0022-3549/82/0100-0050$01.00/0