Ann. Rev. Pharmacol. Toxicol. 1981. 21:265–93

ETHACRYNIC ACID AND RELATED DIURETICS: Relationship of Structure to Beneficial and Detrimental Actions

❖6796

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INTRODUCTION

Over the years it has become evident that the beneficial and detrimental effects of diuretic agents including the phenoxyacetic acid-type (POA acid-type) are multifaceted. For example, the renal effects of a given POA acid-type diuretic might include alteration of sodium chloride and urate transport, changes in renal hemodynamics, intrarenal release of renin and certain prostaglandins, and perhaps a modification of the actions of ADH and kinins. Obviously the gathering of such detailed information about a given drug requires considerable time, and only a relatively small number of POA acids screened are ever studied in such depth. Literally hundreds of newly synthesized POA acids have been screened in a general fashion and their diuretic activity rated on a scale of zero to six, with six being the most effective (1). However, it becomes treacherous to attempt to draw firm relationships between the structure of many of these agents and their renal and extrarenal actions because (*a*) the majority of the agents screened have not been evaluated for much more than their effects on urine flow rate and electrolyte excretion, and (*b*) relatively small numbers of animals are used in many instances. I intend to briefly summarize the relationships between the structures of the POA acids and their diuretic-saluretic actions with special emphasis on studies that have yielded the most quantitative results. Thereafter, a number of the more extensively studied agents are analyzed as to the relationship of their structure to their (*a*) effects on renal hemody-

0362-1642/81/0415-0265$01.00

namics, (*b*) diuretic-saluretic effects, (*c*) ability to alter intrarenal levels of renin and certain prostaglandins, (*d*) effects on urate transport, (*e*) ototoxicity, and (*f*) antihypertensive effects. In addition, areas in which additional experimentation is necessary are cited throughout the review.

RELATIONSHIP OF POA ACID STRUCTURE TO DIURETIC-SALURETIC ACTION: A GENERAL OVERVIEW

The 2,3-dichloro POA acid moiety of POA acid-type diuretics represents the optimal structural requirements for that portion of the molecule (1) (Figure 1). The 4-position of 2,3-dichloro POA has been substituted with a host of different chemical groups. Initial studies in this area were centered around the design of an agent that would react with sulfhydryl-containing substances like the organomercurial merbaphen but would lack the potential toxicity of the mercury atom. Optimal diuretic activity was found in the synthesis of ethacrynic acid (EA) (Figure 1*a*) (2). EA is an avid alkylating agent, and its reactivity with sulfhydryl-containing substances has been demonstrated in vitro (3–5) and in vivo (6).

Many studies tended to support the notion that the sulfhydryl reactivity of a POA acid like EA was an essential structural feature for diuretic-saluretic activity. First, the replacement of the two vinyl hydrogens on EA with methyl groups yielded a compound that was devoid of both sulfhydryl reactivity and diuretic-saluretic activity (Figure 1*b*) (7). Second, the synthesis of POA acid derivatives with simple, nonsulfhydryl reactive acyl groups in the 4-position such as dihydroethacrynic acid (EA-H_2) (8), an epoxide derivative of EA (EAO) (9), and the 4-propionyl derivative (10) proved to possess little or no diuretic-saluretic activity (Figure 1*c*). It was established in each case that the potential diuretic activity of these agents was not masked by a marked reduction in the glomerular filtration rate (GFR). The possibility that the relative inactivity of EA-H_2 might be due to the selective hepatic extraction of the drug was ruled out by administering EA-H_2 to dogs with the cystic and common bile ducts ligated (8). The results paralleled those obtained with the bile and cystic ducts intact. Third, a number of thiol and amine adducts of EA were synthesized (3, 4, 11) (Figure 1*d*) and preliminary screening of these agents revealed that their diuretic effectiveness ranged from those that were totally devoid of diuretic properties to those that were as effective as EA. It would appear that these analogues, being devoid of an α,β-unsaturated ketone moiety, would all lack reactivity toward sulfhydryl-containing substances as well as diuretic-saluretic activity. However, extensive in vitro and in vivo studies demonstrated that the diuretic activity of these adducts was most likely associated with their

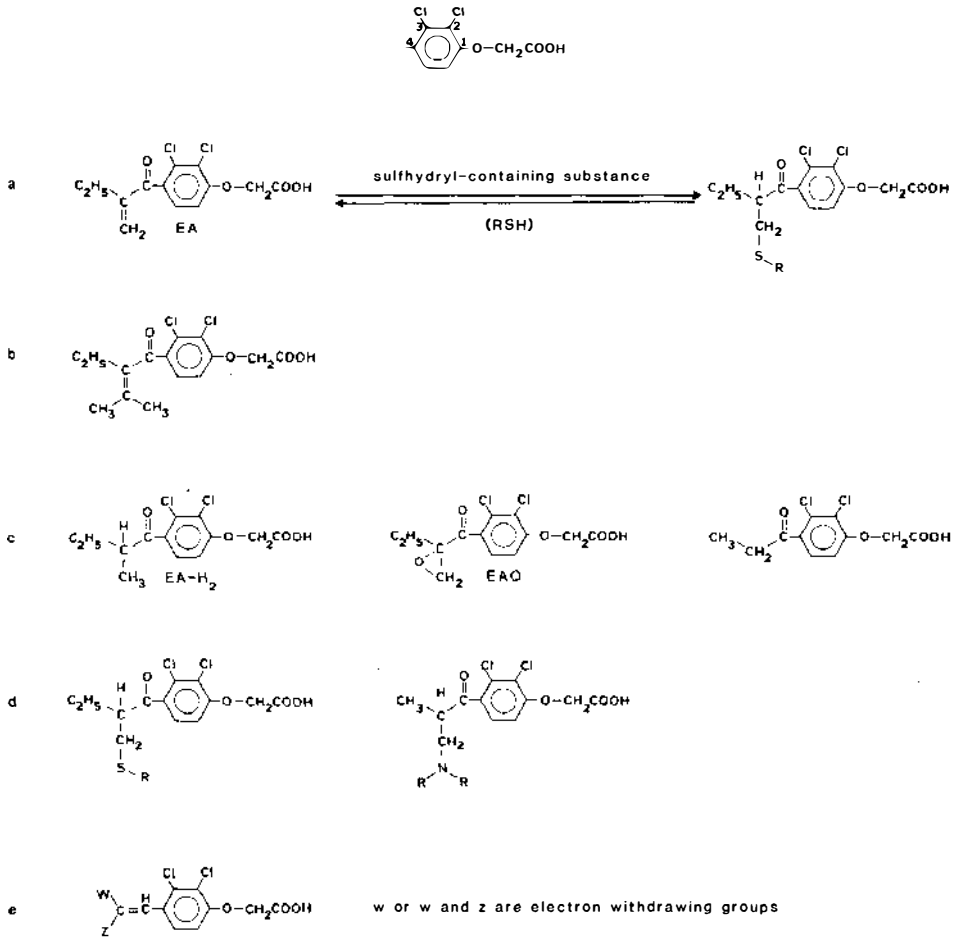

Figure 1 4-Substituted derivatives of 2,3-dichloro POA acid where their sulfhydryl reactivity seemed to parallel their diuretic-saluretic activity.

ability to liberate the parent EA and the corresponding thiol or amine (5, 12, 13). Fourth, certain (vinylaryloxy) acetic acids (Figure 1*e*) were prepared and found to possess diuretic-saluretic properties. Preliminary screening revealed that their diuretic effectiveness roughly paralleled their in vitro reactivity toward sulfhydryl-containing substances (14–17).

However, within the last several years, sulfhydryl reactivity of the POA acid-type diuretic agents has been shown to be relatively unimportant for the expression of diuretic-saluretic properties. First, it was demonstrated that several series of compounds closely related to the POA acid-type

diuretics were completely devoid of sulfhydryl reactivity, yet they were effective diuretic-saluretic agents (Figure 2*a*) (18–20). One of these agents, MK-196 (indacrinone), is currently undergoing clinical trials. Second, a series of relatively simple 4-acyl POA acids was synthesized in which the acyl moiety contained various heterocyclic groups (21). It has been stated that this series of POA acids lacks reactivity toward sulfhydryl-containing substances (22), yet ticrynafen (tienilic acid, SKF 62698, ANP 3624) emerged as a promising diuretic-saluretic agent (Figure 2*b*). Recently ticrynafen was withdrawn from the market because of its suspected hepatotoxicity. Third, Koechel & Rankin (10) synthesized a series of 4-substituted α-bromoketone derivatives of 2,3-dichloro-POA acid (Figure 2*c*) that possessed the capability of reacting at different rates with sulfhydryl-containing substances. However, these POA acids are capable of reacting with such substances by a substitution reaction rather than by a Michael-type reaction as was the case with EA. Theoretically when $R^1 = R^2 = H$, the α-bromoke-

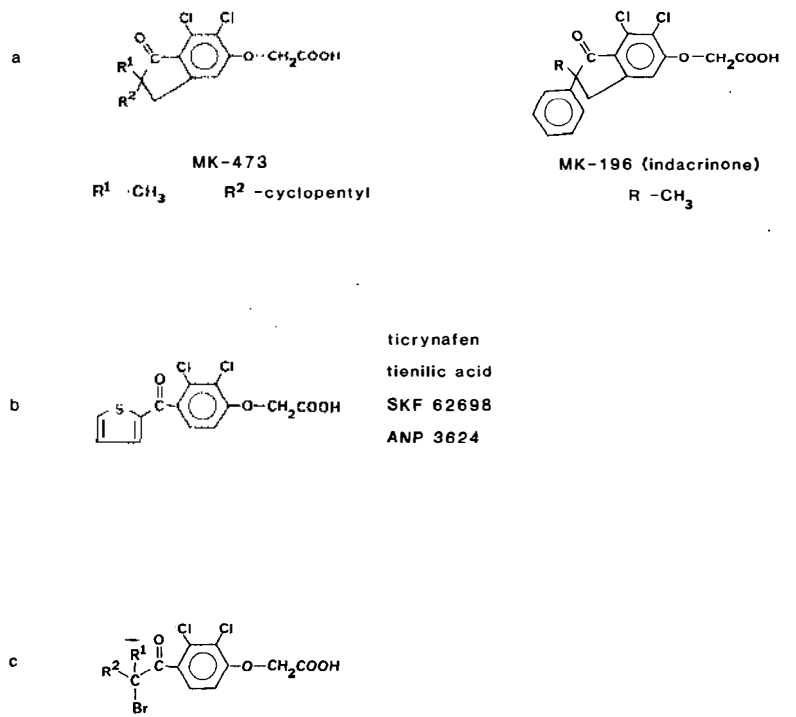

Figure 2 POA acid or POA acid-like compounds that either do not react with sulfhydryl-containing substances (*a* and *b*), or where their sulfhydryl reactivity does not parallel their diuretic-saluretic properties (*c*).

tone should be highly reactive. Whereas when $R^1 = R^2 = CH_3$, the reaction with substances that contain a sulfhydryl group should be very slow or nonexistent. There was no correlation between the anticipated sulfhydryl reactivity of these derivatives and their diuretic-saluretic activity in dogs (10).

Of the hundreds of POA acid-type agents with diuretic-saluretic properties, EA, MK-196 (indacrinone), ticrynafen, and several other related compounds have been studied in more detail than all others. Hence, more reliable data are available concerning the relationship of their structure to their physicochemical properties, plasma protein binding, renal handling, biotransformation, renal actions and extrarenal actions than is the case with the other POA acids. The remainder of the review is devoted to these agents.

RELATIONSHIP OF POA ACID STRUCTURE TO PLASMA PROTEIN BINDING, RENAL HANDLING, AND BIOTRANSFORMATION

EA, MK-196, and ticrynafen are all weakly acidic compounds with similar pKs [EA, 3.5 (23); MK-196, 3.66 (24); ticrynafen, 3.44 (25)]. Their partition coefficients reflect that they are all quite lipid soluble, readily diffusible drugs (23–25). The chemical structure of these POA acids dictates the degree of protein binding, their renal handling, and the mode(s) of biotransformation.

The binding of EA, MK-196, and ticrynafen to plasma proteins (most probably as organic anions) is extensive. Although the actual percentage of EA that becomes bound to plasma proteins has not been established, it is undoubtedly quite high. Ticrynafen is bound to the extent of 97% (25, 26), and 99% of MK-196 is bound to plasma proteins (24).

In general, the renal handling of EA, MK-196, and ticrynafen is very predictable of compounds that (*a*) are extensively bound to plasma proteins, (*b*) are weak acids, and (*c*) possess good lipid solubility. For example, extensive plasma protein binding of these three POA acids severely limits the amount of each drug that enters luminal fluid by glomerular filtration. However, active proximal tubular secretion has been demonstrated for all three of these anionic diuretic-saluretic agents (23, 24, 26–28), and in each case the tubular secretion is subject to competitive inhibition by other organic anions. The active tubular secretion of EA in dogs is reduced by probenecid (23), ticrynafen secretion in dogs is blocked by *p*-aminohippuric acid and salicylates (25), and the active secretion of MK-196 in the chimpanzee is reduced by *p*-aminohippuric acid and probenecid (24). When the urine of dogs or chimpanzees is made alkaline by the administration of $NaHCO_3$, there is an increase in the renal clearance of all three of these

weakly acidic diuretics (23, 24, 26). Thus, because of their physicochemical properties, EA, MK-196, and ticrynafen are all subject to classical carrier-mediated proximal tubular secretion with subsequent passive pH-dependent back diffusion in more distal areas of the nephron.

The variation in the chemical structure of these three diuretics dictates that their main route of biotransformation is different. The biotransformation of EA, like many other highly reactive alkylating agents, involves the formation of a glutathione conjugate which is subsequently degraded to the cysteine and mercapturic acid conjugates (23, 29) (Figure 3). This conversion may occur in the liver or within the renal proximal tubular cells (30, 31). Since ticrynafen lacks a highly reactive functional group, the major route of biotransformation in dog and man is not conjugation with glutathione, but rather the reduction of its ketone group to the corresponding alcohol (32, 33). In addition, a small amount of 2,3-dichloro-4-carboxyphenoxyacetic acid is formed. Relatively little biotransformation of MK-196 occurs in the rat, dog, or monkey (34). However, in the chimpanzee and human more extensive biotransformation occurs with the major metabolite being the *p*-hydroxylation product and minor products resulting from the reduction of the ketone group, methylation of the *p*-hydroxy group, and additional phenyl ring hydroxylation (35).

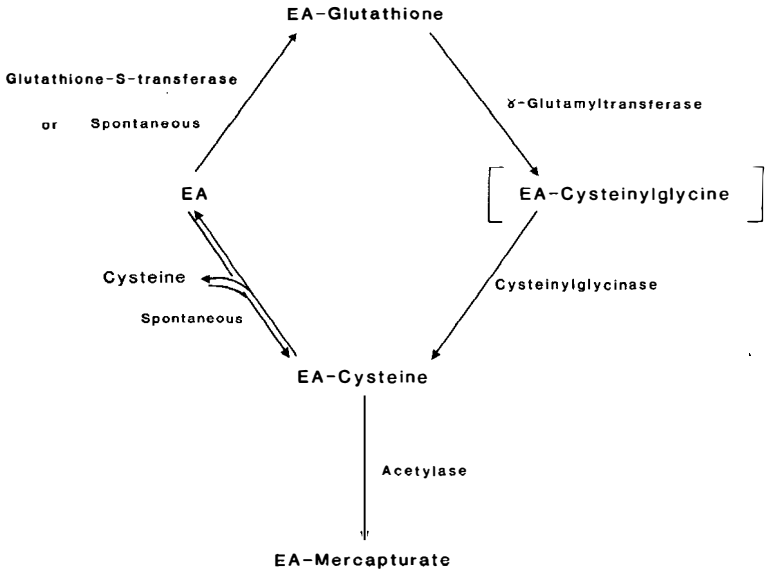

Figure 3 Interconvertibility of the biotransformation products of EA. All of the substances shown have been identified in dog urine or bile except EA-cysteinylglycine. The latter substance may be one of the components of the fraction containing unidentified biotransformation products obtained by a number of investigators (23, 29, 30).

RELATIONSHIP OF POA ACID STRUCTURE TO RENAL HEMODYNAMIC EFFECTS

EA reduces renal vascular resistance in the dog under a variety of conditions where renal blood flow (RBF) and renal perfusion pressure have been closely monitored (36–40). Several reports have noted that the increase in RBF occurs within 2–3 min following the administration of EA into the renal artery or intravenously and is usually preceded by a small transient vasoconstriction (37, 40, 41). Coincident with the increase in total RBF is a redistribution of flow, increasing the proportion to the middle but not to the juxtamedullary cortex (38).

The mechanism by which EA produces renovasodilation is due, at least in part, to the elevation of the intrarenal levels of certain prostaglandins. This is thought to be accomplished by (*a*) an EA-induced intrarenal liberation of certain prostaglandins and (*b*) the inhibition of prostaglandin degradation within the kidney. Williamson et al (41) were the first to demonstrate that the EA-induced increase in RBF in dogs could be markedly reduced by pretreatment with indomethacin, a well-known inhibitor of prostaglandin synthetase (cyclooxygenase). Second, Williamson et al (42) reported that EA simultaneously increased RBF and the renal venous level of prostaglandin E (i.e. $PGE_1 + PGE_2$) but not prostaglandin F. In addition, pretreatment with indomethacin obliterated both the increase in RBF and the increase in renal venous prostaglandin E levels. Third, Patak et al (43) noted that EA simultaneously increased RBF and the renal excretion rate of prostaglandin E (i.e. $PGE_1 + PGE_2$) in hydropenic dogs. Under similar conditions certain other classes of diuretics, such as chlorothiazide and the carbonic anhydrase inhibitor benzolamide, failed to change the renal excretion rate of prostaglandin E or markedly alter RBF. In spite of this finding, Kirschenbaum & Serros (44) demonstrated that urinary prostaglandin E excretion rates are determined to a great extent by urine flow rate and questioned the significance of the interpretation of elevated prostaglandin E excretion rates during certain diuretic states. However, Work et al (45) recently reported that the urinary excretion rate of prostaglandin E was not influenced by urine flow rate. Fourth, if the prostaglandins are involved in the EA-induced increase in RBF, then arachidonic acid as well as certain prostaglandins themselves may be expected to produce EA-like hemodynamic changes when injected into the canine renal artery. Tannenbaum et al (46) found that the infusion of sodium arachidonate into the canine renal artery increased RBF, and that the increase could be abolished by blockade of prostaglandin synthetase. It has also been shown that prostaglandin E_2 (46–54), prostacyclin (PGI_2) (50, 51, 55), prostaglandin D_2 (50, 51), and prostaglandin A_1 (48) all increase RBF in the dog following their injection into the renal artery under conditions when there is no change in the renal

perfusion pressure. Prostaglandin $F_{2\alpha}$ (46, 48, 49), and 6-keto-prostaglandin $F_{1\alpha}$ (a metabolite of prostacyclin) (50) do not alter canine renal hemodynamics. In addition, arachidonic acid (49), prostaglandin E_2, prostaglandin D_2, and prostacyclin all produce a redistribution of RBF away from the outer cortex (zone 1) and toward the juxtamedullary cortex (zone 4) (55). The effect of prostacyclin on renal hemodynamics is extremely interesting since it is one of the few prostaglandins that is synthesized by renal cortical microsomes (56–58). Unfortunately, the effect of EA on renal venous levels or urinary levels of prostacyclin or its major biotransformation product—6-keto-prostaglandin $F_{1\alpha}$—has not been investigated.

The EA-induced elevation of renal prostaglandin levels is thought to be due primarily to an increase in the intrarenal synthesis of these lipids. However, this is mostly by inference because furosemide, like EA, increases both total RBF and the renal excretion rate of prostaglandin E, and it has been shown to do so by enhancing the synthesis and intrarenal release of prostaglandins (59).

EA probably also elevates the intrarenal levels of certain prostaglandins by inhibiting 15-hydroxyprostaglandin dehydrogenase and prostaglandin E-9-keto-reductase, the two major enzymes responsible for their intrarenal degradation. Although the inhibition of these two enzymes is apparently quite species dependent (60, 61), EA has been shown to inhibit 15-hydroxyprostaglandin dehydrogenase isolated from rabbit, rat, human, and guinea pig kidney (61–63) as well as from human placenta (64). EA also inhibits the 9-keto-reductase in rabbit, rat, and human kidney (1, 61).

The hemodynamic effects of ticrynafen and MK-196 have not as yet been published in detail. However, preliminary studies have shown that ticrynafen has little effect on renal hemodynamics (65, 66). Several reports have cautioned against using *p*-aminohippuric acid to determine effective renal plasma flow unless the renal extraction of *p*-aminohippuric acid is estimated because ticrynafen dramatically reduces the active tubular secretion of *p*-aminohippuric acid (25, 65). MK-196 has been shown to inhibit both of the major prostaglandin metabolizing enzymes in lower concentrations than were required for EA (1). It is not known what effect MK-196 has on total RBF or on the intrarenal release of prostaglandins.

At the present time the dog is one of the best animal models for investigating the hemodynamic and diuretic-saluretic effects of the POA acid-type agents. Unless an investigation is being conducted to delineate reasons for species variations the rat represents a particularly poor animal model for studies involving EA. The rat (*a*) is relatively resistant to the diuretic effect of EA (23, 67), and (*b*) in contrast to the vasodilation observed in the dog, prostaglandin E_2, prostaglandin D_2, and arachidonic acid are renovasoconstrictor agents in the rat (68, 69).

RELATIONSHIP OF POA ACID STRUCTURE TO DIURETIC-SALURETIC ACTIVITY

Small changes in the structure of the POA acids cause very important changes in their anatomical site of diuretic-saluretic action within the nephron, the pattern of their diuretic-saluretic response, and perhaps the mechanism responsible for the diuretic-saluretic response.

Relationship of POA Structure to Site of Saluretic Action

EA is thought to inhibit (*a*) active chloride transport in the medullary thick ascending limb of Henle (MTALH) (also referred to as the medullary diluting segment), and in the cortical thick ascending limb of Henle (CTALH) (also referred to as the cortical diluting segment), and (*b*) active sodium transport in the cortical collecting tubule (CCT). The foregoing conclusions were arrived at by five different types of studies. First, routine clearance studies have shown that EA produces a decrease in C_{H_2O} during water loading and a decrease in $T^{c'}_{H_2O}$ during hydropenia in humans (70, 71) and dogs (23, 72–74). These findings indicate that EA may alter electrolyte transport in the CTALH and in the MTALH. Second, EA also abolishes the medullary sodium/urea gradient which is indicative of an action on the MTALH (23). Third, Schnieders & Ludens (75) investigated the effect of EA on the toad bladder (which is analogous to the late distal tubule and collecting duct in that it actively transports sodium from the urinary side to the blood side of the epithelium) and the toad cornea (which is analogous to the thick ascending limb of the loop of Henle in that it actively transports chloride). They found that EA reduced the short circuit current in both preparations. Fourth, the most direct evidence for the anatomical site of action of EA resulted from the studies of Burg & Green (76). These investigators found that EA inhibited the active transport of chloride in the isolated perfused rabbit CTALH. However, active chloride transport occurs in both the CTALH and the MTALH, and it is now known that these two segments of the nephron are anatomically and functionally different (77). Therefore, similar experiments should be conducted on isolated perfused rabbit MTALH to ascertain whether EA also inhibits active chloride transport in this segment of the nephron. Fifth, Abramow (78) has demonstrated that EA antagonizes the action of ADH in the isolated perfused CCT of the rabbit nephron.

Ticrynafen has been shown by clearance studies in dogs (28) and man (79–81) to decrease C_{H_2O} during water loading but has little or no influence on T_{H_2O} during hydropenia. It has been concluded that ticrynafen and the thiazide diuretics share the same anatomical site of action within the nephron. Unfortunately, this site of action has been referred to repeatedly as the

cortical diluting segment of the distal tubule. This terminology has resulted in some confusion since Jacobson & Kokko (82) concluded that the thiazides act on the cortical diluting segment which is equivalent to the CTALH (a segment that is conceived to begin at the corticomedullary junction and extend to the macula densa).

The possibility that EA and the structurally related ticrynafen share the CTALH as a site of diuretic-saluretic action, with only EA acting at the MTALH, is one of the most interesting findings reported in this review. It demonstrates that, like the sulfamoyl-containing diuretics (thiazides versus furosemide), small changes in the POA acid structure can create drugs that act at some anatomical sites within the nephron that are not shared by other POA acids.

Clearance studies in rats (83), micropuncture studies in rats (84), and studies on the toad bladder and cornea (75) indicate that MK-196 acts primarily on the thick ascending limb of Henle's loop and perhaps a secondary site of action at the level of the collecting tubules. More direct experiments utilizing the in vitro microperfusion of isolated rabbit tubles have not been reported. These preliminary data support the notion that perhaps EA and MK-196 act at similar anatomical locations within the nephron, whereas ticrynafen may be importantly different in this regard.

Relationship of POA Acid Structure to the Pattern of the Diuretic-Saluretic Action

EA induces a marked diuresis-saluresis in most all animal species except the rat (23, 67). Most aspects concerning the renal effects of EA have been investigated in dogs because the dog responds to EA in much the same fashion as the human. Dose-response data in dogs (12, 23) reveal that EA elicits a diuresis-saluresis following intravenous administration that is rapid in onset, high-ceiling, and of relatively short duration. The peak diuretic response is usually observed within 15 to 20 min and is of such a magnitude as to result in the excretion of 17–25% of the filtered load of sodium (72). It has been shown repeatedly that the onset of the EA-induced diuresis occurs at the same time that this agent increases RBF. However. it is unknown what role the EA-induced change in renal hemodynamics plays in the net diuresis.

The natriuretic and diuretic properties of ticrynafen and EA have been compared in dogs under a variety of conditions (28, 65). Ticrynafen differs from EA in its efficacy, duration of action, and site of natriuretic activity. Whereas EA may induce the loss of approximately 19% of the filtered load of sodium, ticrynafen is more like the thiazides in that it induces the loss of 3–10% of the filtered load of sodium. In addition, ticrynafen seems to have a longer duration of action than EA in this species. Lemieux et al (65) also demonstrated that ticrynafen is diuretic in the rat.

The diuretic-saluretic activity of MK-196 has been investigated in the dog, rat (84), and chimpanzee (24, 85, 86). Although the rat is not as resistant to the renal effects of MK-196 as it is to those of EA, it still requires relatively high doses of MK-196 (i.e. 50 mg/kg) to elicit a diuretic response in this species (84). We have observed that the MK-196-induced diuretic-saluretic response in dogs given intravenous doses between 3.3 and 17 μmol/kg is highly variable and is of lower magnitude than that observed with equivalent doses of EA (D. A. Koechel, unpublished observation). This observation apparently does not apply to man. At this time a significant amount of the published work dealing with the diuretic-saluretic action of MK-196 has been conducted in the chimpanzee because MK-196 also has uricosuric activity, and the chimpanzee is the best species in which to evaluate this latter type of drug activity (24, 85, 86). Unfortunately, it is difficult to draw firm conclusions based on the chimpanzee data because (*a*) a relatively small number of animals was used in the studies, and (*b*) the dose-response studies were conducted on chimpanzees ranging in weight from 22 to 79 kg; however, the weights of the chimpanzees were not presented along with the excretion data. Nevertheless, MK-196 appears to have a rapid onset of action much like EA and a longer duration of diuretic-saluretic action (86).

Relationship of POA Acid Structure to the Mechanism of Diuretic-Saluretic Action

Inhibition of the renal NA^+,K^+-ATPase, adenyl cyclase, and inhibition of renal glycolysis have been suggested as possible mechanisms by which EA and related diuretics might induce an increase in the renal excretion of water and electrolytes. These topics have been reviewed in the recent past and are not expanded upon here (76, 82). Interestingly, the effects of MK-196 and ticrynafen on these enzyme systems have not been reported.

Previously we emphasized that some of the POA acid-type diuretics, EA being the prototype, induce a rather rapid intrarenal release of certain prostaglandins which is thought to be responsible for the observed change in renal hemodynamics. Prostaglandins may also contribute, in part, to the diuretic-saluretic response of certain of the POA acid-type agents—at least those capable of increasing the intrarenal levels of key prostaglandins (i.e. PGE_2 and PGI_2). Several important findings support such a role for certain prostaglandins. First, prostaglandin E_2 (46, 48, 50, 54, 87), prostaglandin $F_{2\alpha}$ (46, 48, 87), prostacyclin (PGI_2) (50, 55), and the prostaglandin percursor, arachidonic acid (46, 88), have been shown to produce a dose-dependent natriuresis and diuresis when injected into the canine or rabbit renal artery. On the other hand, prostaglandin D_2 (50), prostaglandin A_1 (48), prostaglandin A_2 (87), and 6-keto-prostaglandin $F_{1\alpha}$ (a biotransformation product of prostacyclin) (50) are devoid of any effect on urine flow rate or

electrolyte excretion. Second, it has recently been shown that certain prostaglandins inhibit the active transport of chloride in the MTALH, as well as the active sodium transport that occurs in the cortical and outer medullary collecting tubule. When the isolated perfused rabbit MTALH is exposed to prostaglandin E_2 in the bath or the perfusate, a fall in net chloride transport of 40–50% was observed with a concomitant fall in voltage (77). In contrast, prostaglandin E_2 did not affect net chloride transport in the CTALH when it was introduced into the bath or the perfusate (77). This finding further emphasizes the fact that the CTALH and the MTALH are functionally different (77). Third, prostaglandins have also been shown to inhibit active sodium transport in isolated rabbit CCT (89, 90). Abramow (78) implicated this segment of the nephron as a site affected by EA. When prostaglandin E_2 was applied to the bath, it rapidly inhibited the net sodium transport out of the lumen of the CCT and the outer medullary collecting tubule. No effect was observed when prostaglandin E_2 was applied in the perfusion solution. Prostaglandin $F_{2\alpha}$ inhibits net sodium transport out of the CCT when applied to the bath, whereas similar application of prostaglandin A_2 has no effect (89, 90).

Since EA causes the intrarenal release of certain prostaglandins and its anatomical sites of saluretic action are similar to those of certain prostaglandins it is tempting to speculate that EA may be acting, in part, through the action of certain prostaglandins. For the purpose of relating the structure of the POA acid-type diuretics to their diuretic-saluretic action, ticrynafen and MK-196 should be examined for their ability (*a*) to increase the renal venous and urinary levels of certain prostaglandins (currently prostaglandin E_2 and I_2 appear to be the most important) and (*b*) to alter active chloride and sodium transport in isolated perfused segments of rabbit tubules.

RELATIONSHIP OF POA ACID STRUCTURE TO THE ACUTE RELEASE OF RENIN

Diuretic agents may induce an increase in plasma renin activity (PRA) on two different time scales. First, the chronic use of all diuretics, regardless of their chemical structures or their anatomical sites of action within the nephron, will induce salt and water losses that will eventually modify the volume and composition of the extracellular fluid and diminish the renal perfusion pressure. These changes will trigger the hypersecretion of renin. Second, EA and certain other diuretic-saluretic agents are capable of producing an immediate hypersecretion of renin that is independent of variations in extracellular volume and is associated with an intrarenal mechanism.

In general it is postulated that renin is released into the plasma by (*a*) an intrarenal vascular "baroreceptor" in the afferent arterioles that senses

changes in arterial pressure or wall tension and releases renin in response to decreased pressure, (*b*) a β-adrenergic receptor that releases renin when stimulated by catecholamines such as isoproterenol, and (*c*) the macula densa mechanism, mediated by specialized cells in the distal tubule that are sensitive to changes in either intraluminal concentrations of sodium chloride or transport of one or both of these ions into the macula densa itself (91). Thames & DiBona (92) recently demonstrated that there is an interaction between these three renin-secreting processes.

It has been concluded from a number of studies that EA stimulates an immediate release of renin by an effect on the macula densa mechanism (93–97) and also by an effect on the baroreceptor mechanism (98). Unfortunately there have been no reports concerning the effects of MK-196 or ticrynafen on the acute release of renin. The influence of chronic administration of ticrynafen in humans has resulted in an increase in PRA that has been associated with a reduction in plasma volume (99, 100).

Evidence has accumulated that suggests the prostaglandins are intimately involved in the control of renin secretion (88, 101–104). Recently it has been demonstrated that the prostaglandin system (especially prostaglandins E_2 and I_2) stimulates the release of renin via the baroreceptor mechanism (105–107).

It is undoubtedly significant that the only diuretics known to produce an immediate increase in PRA are those drugs that (*a*) increase the renal venous and urinary levels of prostaglandin E, (*b*) increase RBF, and (*c*) are thought to inhibit ion transport in the MTALH. Such agents include EA (93–98) and the aminobenzoic acid-type diuretics furosemide (108, 109) and bumetanide (110). Whereas those diuretics that act at sites other than the MTALH such as chlorothiazide (93), metolazone (96, 97), clopamide (96), and indapamide (96) do not induce an immediate release of renin. It is known that chlorothiazide does not increase RBF or the urinary excretion rate of prostaglandin E (43).

Since it has been shown that certain prostaglandins stimulate the baroreceptor mechanism leading to the immediate release of renin, it seems most appropriate to use the elegant procedure described by Data et al (105) and Gerber et al (107) to assess the ability of a POA acid-type diuretic to acutely elevate plasma renin levels by the baroreceptor mechanism.

RELATIONSHIP OF POA ACID STRUCTURE TO URICOSURIC ACTIVITY

While the dog is a notoriously poor responder to uricosuric agents (111, 112), the chimpanzee is one of the best animal models to use for studying the effect of drugs on the renal excretion of urate. Like man, the chimpanzee

lacks uricase, maintains relatively high levels of plasma urate, and has a similar C_{urate}/C_{inulin} (approximately 0.1). Fanelli & Weiner (113) have cautioned that the effect of a drug on the renal handling of urate in the chimpanzee may be only suggestive of the effect expected in man because the chimpanzee is a hyperresponder to uricosuric drugs.

There is a marked variation in the chemical structures of agents that produce a uricosuric response by inhibiting the proximal tubular reabsorption of urate. For example, *p*-aminohippuric acid, probenecid, sulfinpyrazone, acetoheximide, and certain radio-contrast agents make up a long list of drugs with uricosuric properties (114). It appears that most of the drugs that induce a uricosuric response possess a weakly acidic functional group, yet exceptions to this generalization exist (115–117). Unfortunately, the presence of a weakly acidic functional group may also lead to a compound that may cause urate retention as a result of blocking the proximal tubular secretion of urate. Another issue that complicates structure-activity relationship studies in this area surfaces if the acidic drugs under investigation simultaneously alter the renal handling of urate and electrolytes. In such cases, urate retention may occur because (*a*) the weakly acidic drug may competitively inhibit the proximal tubular secretion of urate (118, 119) or (*b*) the diuretic response may lead to a reduction in the extracellular volume which in turn may lead to urate retention (120, 121). If an attempt is made to relate the chemical structure to the uricosuric activity within a class of drugs that also possesses diuretic activity and if drug-induced volume contraction can ultimately lead to urate retention, then one must attempt to design a drug molecule that has good uricosuric activity but not excessive diuretic activity.

Relatively small changes in the structure of the POA acid-type diuretics have resulted in compounds with varying effects on the renal handling of uric acid. For example, the effect of EA on the renal handling of urate was found to be biphasic. Preliminary studies in the chimpanzee revealed that EA (1 mg/kg, i.v.) induces a very transient uricosuria within 15 min (86). A similar effect was not noted in dogs (65). When EA was administered intravenously to humans, the clearance of urate increased in most subjects. However, more prolonged oral administration of EA to humans produced hyperuricemia and diminished uric acid excretion in some individuals (122–124). These early results were explained on the basis that the lower blood levels of EA that result from prolonged oral administration of EA inhibit proximal tubular secretion of urate, whereas the higher blood levels of EA that result from intravenous therapy inhibit urate reabsorption. More recent findings indicate that EA-induced reduction in plasma volume may also have contributed to the urate retention (120, 121).

Ticrynafen, on the other hand, produces frank hypouricemia as well as

a mild diuretic-saluretic response. The hypouricemic effect of ticrynafen occurs solely as a result of its ability to block the proximal tubular reabsorption of urate. Stote et al (125) showed that ticrynafen does not reduce plasma urate levels as a result of an inhibition of xanthine oxidase, and Murray et al (126) found that the administration of ticrynafen to anephric patients did not alter the serum urate concentrations. This latter observation points to the importance of the functioning kidney for the hypouricemic effect of ticrynafen.

It has been shown that the diuretic-saluretic and uricosuric effects of ticrynafen occur as the result of an action of the drug at different anatomical sites within the nephron. First, stop flow studies in dogs revealed that ticrynafen blocks net urate reabsorption in the proximal segment of the nephron (65), whereas clearance studies with ticrynafen have demonstrated that its major site of natriuretic action is in the cortical diluting segment of the ascending limb of the loop of Henle (28, 79–81). Second, the diuresis but not the uricosuric effect is seen in guinea pigs, rabbits, and chickens (65). Third, pyrazinamide diminishes the uricosuric response to ticrynafen in humans at a time when it has no effect on the natriuretic response (66, 127, 128). Fourth, Prasad et al (127) observed that the renal excretion of sodium and urate did not increase in parallel in humans receiving single daily doses of 500 mg of ticrynafen.

Lemieux et al (65) demonstrated that ticrynafen was uricosuric in species that normally show net urate reabsorption (i.e. $C_{urate}/C_{inulin} < 1$) such as the non-Dalmatian dog, rat, and guinea pig, but was actually antiuricosuric in species that show net urate secretion (i.e. $C_{urate}/C_{inulin} > 1$) such as the rabbit and chicken. Interestingly, ticrynafen is the most efficacious uricosuric agent known in the non-Dalmatian dog (65). When ticrynafen was administered orally to chimpanzees in a dose of 1 mg/kg, a modest uricosuric effect occurred that was not accompanied by changes in electrolyte excretion (129).

Human studies with ticrynafen have revealed that (*a*) the observed uricosuric effect is most likely due to ticrynafen itself and not the result of biotransformation products (128), (*b*) during several 12-week double-blind crossover studies, the uricosuric activity of 125–250 mg/day of ticrynafen was comparable to that of 500–1000 mg/day of probenecid (130–132), and (*c*) the balance of diuretic-saluretic and uricosuric properties is weighted in favor of the uricosuric properties. A marked uricosuria occurs with very little or no reduction in the plasma volume in studies that have involved daily administration of ticrynafen for as long as 24 weeks (99, 133–136). When ticrynafen therapy is stopped, the plasma levels of urate return to predrug levels within one week. The marked uricosuric properties and the relatively weak diuretic properties of ticrynafen have undoubtedly con-

tributed to the urate nephropathy observed with this agent (131, 132, 137–141).

Like ticrynafen, MK-196 and MK-473 [(2-cyclopentyl-6,7-dichloro-2-methyl-1-oxo-5-indanyloxy)acetic acid] (Figure 2) possess diuretic-saluretic and uricosuric properties. Preliminary acute experiments in the chimpanzee revealed that both of these agents induce a dose-dependent uricosuria, and that MK-196 is more efficacious in this regard than MK-473 (85, 86). The maximum uricosuric effect of MK-196 occurred with a dose of 2.5 mg/kg orally. Fanelli et al (24) also demonstrated that the renal sites of MK-196-induced uricosuria and natriuresis are different because pyrazinamide altered the renal handling of urate in the champanzee at a time when the natriuretic response remained unchanged.

When MK-196 was administered in doses of 20 to 80 mg/day to healthy human volunteers, a uricosuric effect was induced but did not last beyond the first 6 hr (142). Thereafter urate retention occurred, so the net change in plasma urate over the 24 hr period of study was minor. In this same study MK-196 induced a diuresis-saluresis and kaluresis that lasted for 12 and 24 hr respectively.

MK-196 has a chiral center, and the individual isomers possess different degrees of diuretic-saluretic and uricosuric properties (1). This allows isomers to be combined in various proportions in an attempt to obtain the desired diuretic-saluretic and uricosuric properties. This is not possible to do with EA or ticrynafen since they do not possess a chiral center.

Recently, the diuretic-saluretic and uricosuric properties of 6,7-dichloro-2,3-dihydro-5-(2-thienylcarbonyl)benzofuran-2-carboxylic acid were reported (129). This agent possesses a chiral center, and the racemic mixture and the *d* isomer exhibit diuretic-saluretic properties in the chimpanzee, dog, and rat. Interestingly, the *l* isomer was uricosuric but devoid of any diuretic-saluretic properties in the chimpanzee and similarly was not diuretic in the dog and rat. Again, it becomes possible to combine the two isomers in appropriate proportions to attain the desired balance of diuretic-saluretic and uricosuric activities. Fanelli et al (129) noted that the diuretic-saluretic activity of the *d* isomer in the chimpanzee and the dog was far greater than that observed for ticrynafen.

It is important to consider that a weakly acidic functional group is frequently an essential portion of a uricosuric agent. However, it is possible that the presence of such a functional group may also produce a compound that induces urate retention. In addition, if the drug also possesses diuretic activity, then urate retention may be induced secondary to extracellular volume contraction. All of these factors are at play with the POA acid-type diuretics.

THE RELATIONSHIP OF POA ACID STRUCTURE TO THEIR OTOTOXICITY

The ototoxic nature of a drug may be signaled by decreases in various electrophysiological potentials such as the endocochlear potential, the N_1 potential, the cochlear microphonics, or by the loss of the Preyer reflex. The study of drug-induced changes in these cochlear potentials has been complemented by studies that involve the use of light and electron microscopy for the examination of drug-induced changes in cochlear morphology.

EA has been shown to produce a dose-dependent loss of the Preyer reflex in guinea pigs (143), a decrease in the endocochlear potential in rats (144) and guinea pigs (145–148), a decrease in the ac cochlear potential in cats (149–151) and guinea pigs (145–147, 150–153), and a decrease in the N_1 potential in cats (150, 154, 155). Decreases in cochlear potentials can be observed within 10 min following the intravenous administration of EA in doses that exceed 30 mg/kg. McCurdy et al (153) demonstrated that a nonototoxic dose of EA (20 mg/kg) in normal guinea pigs decreased the ac cochlear potential in anephric guinea pigs. This parallels the increased ototoxicity of EA in humans with impaired renal function (156). The consensus is that the stria vascularis is the source for the endocochlear potential, and that this is probably the site of the initial EA-induced insult.

In support of the electrophysiological studies it has been shown that EA alters the normal morphology of the stria vascularis in guinea pigs (143, 147, 157–159), cats (150, 151), and rats (144) in a dose-dependent fashion. Light and electron microscopic studies have revealed that doses of EA in excess of 30 mg/kg produce an alteration (edema) of the marginal and intermediate cells of the stria vascularis within 10–30 min following drug administration (143, 147, 150, 158, 159). EA-induced alteration of strial morphology commences at approximately the same time as the maximal decreases in the cochlear potentials are observed. The maximum alteration in strial morphology is usually evident within about 1 hr after EA administration (143, 147, 158, 159). However, the recovery of the strial morphology clearly lags behind the return of the ac cochlear microphonics and the endocochlear potential to pretreatment levels (147). In addition, the outer hair cells become damaged in the guinea pig (143) and the cat (150) as the intravenous dose of EA is increased beyond 50–80 mg/kg. Several reports have also addressed the potential vestibulotoxicity associated with high doses of EA (160, 161).

The mechanism of EA-induced ototoxicity is unknown. It has been suggested that inhibition of the strial Na^+,K^+-ATPase by EA may ultimately lead to changes in cochlear potentials and cochlear morphology (145, 162)

but Kusakari et al (148) demonstrated that such an event was unlikely. EA-induced inhibition of strial adenyl cyclase has also been proposed, but this requires additional support (163).

It has been suggested by some investigators that EA-cysteine may induce the ototoxicity whereas others feel that EA itself is the culprit. The design of new POA acid-type diuretics that lack ototoxic potential may be influenced by the resolution of this controversy. Three observations have implicated EA-cysteine as the ototoxic drug species. First, Schneider & Becker (156) observed that transient hearing loss following EA therapy occurred most frequently in patients with impaired renal function and suggested that it was due to the accumulation of a potentially ototoxic biotransformation product such as EA-cysteine. Second, EA-cysteine (MK-597) was withdrawn from clinical trials because it induced a relatively high incidence of transient hearing loss (156). Third, Brown (154, 155) noted that there was a slight lag in the onset of the ototoxic response to EA but not to EA-cysteine, and suggested that EA must undergo biotransformation to EA-cysteine prior to exerting the ototoxic response.

Fox & Brummett (164) marshaled the idea that EA is the drug species responsible for the ototoxicity. These investigators found that EA was more extensively bound to plasma proteins than EA-cysteine, and that EA-cysteine spontaneously releases an EA-like product. They concluded that the higher plasma concentration of free EA-cysteine would allow more EA-cysteine to reach the cochlear sites where it could then spontaneously liberate EA. Koechel & Cafruny (5, 12) proposed a similar concept to explain why EA-cysteine inhibits active chloride transport in the rabbit CTALH at lower concentrations than EA (76).

The ototoxic potential of EA, dihydroethacrynic acid (EA-H_2), an epoxide of EA (EAO), and five thiol adducts of EA has recently been examined (see Figure 1 for structures) (R. E. Brummett, K. E. Fox, and D. A. Koechel, unpublished). A direct correlation was found between the rate at which the five thiol adducts of EA liberate EA in vitro (5, 12) and their ototoxic potential in guinea pigs. That is, the order of ototoxic potential was EA-cysteine > EA = EA-mercaptoethylamine = EA-glutathione > EA-thiosalicylate>>>>EA-3-mercaptopropionic acid. Although EA-glutathione does not spontaneously release EA in vitro, it is biotransformed to EA-cysteine which can spontaneously release EA (5, 29). Interestingly, the diuretic efficacy of the thiol adducts of EA in dogs (5, 12) parallels their ototoxic potential in the guinea pig. EAO and EA-H_2 were both devoid of ototoxicity in guinea pigs in doses equivalent to 80 mg/kg of EA. Neither EAO (9) nor EA-H_2 (8) elicit much of a change in the excretion rate of electrolytes in the dog.

The ototoxicity of MK-196 has not as yet been reported. However, preliminary studies with ticrynafen have shown that one third of the cats that received an intravenous dose of 135 mg/kg experienced hearing defects. Furthermore, when ticrynafen was administered to guinea pigs in intravenous doses up to 250 mg/kg, bilateral distortions of the outer hair cells occurred (T. Selby, personal communication). A recent clinical study found no evidence of deterioration of hearing following the administration of ticrynafen (mean daily dose of 210 mg) for three months (100).

Several important points emerge from these studies. The first point relates to the importance of the sulfhydryl reactivity of the POA acids for the expression of the ototoxic response. The finding that EA is most likely released from certain thiol adducts of EA before an ototoxic response is observed indicates that the liberation of the highly reactive EA is an essential ingredient of the ototoxic response. In addition, the failure of EAO and EA-H_2 to react with sulfhydryl-containing substances, as well as their apparent lack of ototoxicity, suggests that sulfhydryl reactivity may be an important structural feature of the ototoxic POA acids. However, data from several other sources support the notion that sulfhydryl reactivity of the POA acids is not important for the expression of an ototoxic response. For example, ticrynafen is claimed to be nonreactive (81), yet it is capable of inducing an ototoxic response at relatively high doses. Also, the aminobenzoic acid-type diuretics such as furosemide, bumetanide, and piretanide are unable to react with sulfhydryl-containing substances, yet produce an ototoxic response that closely resembles that induced by EA and EA-cysteine. In regard to the latter point (*a*) furosemide and bumetanide produce changes in the N_1 potential in dogs (165) and in the endocochlear potential in guinea pigs (166) that are similar to those produced by EA, (*b*) the dose-ototoxicity curves for EA, EA-cysteine, furosemide, and bumetanide are all parallel (154, 155, 165), and (*c*) Santi & Duvall (167) recently revealed that EA and bumetanide induce practically identical changes in the cytoarchitecture of the stria vascularis. Thus, if all of these diuretic agents elicit an ototoxic response by the same mechanism then it is highly unlikely that their ability to react with sulfhydryl-containing substances plays a dominant role in the elicitation of the ototoxic response.

Second, there is an unexplained relationship between the kidney and the cochlea insofar as many of the POA acid-type and aminobenzoic acid-type diuretics are concerned. For example, furosemide, bumetanide, piretanide, EA, and presumably EA-cysteine all (*a*) act on the MTALH, (*b*) induce the intrarenal release of renin and certain prostaglandins, (*c*) possess parallel dose-ototoxicity curves, and (*d*) induce similar morphologic changes in the stria vascularis. Other similarities between these two tissues have been

reviewed by Brown & Feldman (168). Thus, future structure-activity relationship studies should attempt to relate the structure and action of one POA acid with the structure and actions of other POA acids, as well as attempt to elucidate what the POA acid-type diuretic agents and the aminobenzoic acid-type diuretics have in common, mechanistically, to evoke similar beneficial and detrimental actions.

RELATIONSHIP OF POA ACID STRUCTURE TO ANTIHYPERTENSIVE ACTIVITY

EA, MK-196, and ticrynafen all possess antihypertensive properties when examined in man. The antihypertensive effect of EA has been compared with that of hydrochlorothiazide in doses that yield an equivalent diuretic-saluretic response (169), as well as on a mg/mg basis (170–172). The results indicate that the two agents are very similar in their antihypertensive activity. Several other studies have attested to the antihypertensive properties of EA (173, 174).

The antihypertensive effect produced by ticrynafen and MK-196 is also comparable to the antihypertensive effect of hydrochlorothiazide. Early studies revealed that a 250 mg dose of ticrynafen produced a natriuresis over a period of 12–14 hr that was comparable to that produced by 50 mg of hydrochlorothiazide (80). Hence, the antihypertensive activity of the two diuretics has been compared utilizing these doses (99, 133–135, 175–185). In all cases the data indicate that there is no major difference in the antihypertensive efficacy of these two agents. Preliminary studies indicated that daily doses of 10–15 mg of MK-196 lower blood pressure as much or more than 50 mg of hydrochlorothiazide (186). Thus, sulfhydryl reactivity of these diuretic-saluretic agents is apparently not a prerequisite for antihypertensive activity.

EA VERSUS EA-CYSTEINE AS THE ACTIVE DIURETIC-SALURETIC, OTOTOXIC SPECIES

During the initial work with EA the assumption was made that its renal effects were most likely the result of its reaction with renal receptors (2). More recently, Burg & Green (76) proposed that EA-cysteine is the actual diuretic-saluretic drug species, and Brown (154, 155) has fostered the notion that this species is also responsible for the ototoxicity associated with EA. For several reasons this reviewer feels that it is premature to invoke EA-cysteine as the drug species responsible for the renal and otic effects following the administration of EA. First, it has been demonstrated repeatedly that the in vitro reaction between EA and cysteine occurs extremely

fast (i.e. $T_{1/2}$ = 0.8 min) (7), and perhaps even more important for this discussion is the awareness that the reverse reaction (i.e. the release of EA and cysteine from EA-cysteine) has been shown to occur rapidly under specific in vitro conditions (5, 12). These observations illustrate the tremendous interconvertibility of EA and EA-cysteine. EA reacts with many other sulfhydryl-containing substances, and the rate of the reverse reaction varies depending on the chemical nature of the sulfhydryl-containing substance (5, 12). Thus, following the introduction of EA into the body, one would anticipate that the amount of free EA available to renal or cochlear sites will be highly dependent on the rate that it reacts with various plasma and tissue substances and the rate that it is released from such covalent binding. Second, biotransformation products other than EA-cysteine also possess diuretic-saluretic and ototoxic properties (Figure 3). Koechel & Cafruny examined the diuretic-saluretic properties of EA and EA-cysteine (5, 12) as well as EA-glutathione (unpublished observation) and found that they were all capable of eliciting a marked diuretic-saluretic response. In addition, R. E. Brummett, K. E. Fox, and D. A. Koechel recently observed a similar degree of ototoxicity following the administration of EA, EA-cysteine, and EA-glutathione to guinea pigs (50 mg/kg) (unpublished observation). Of the remaining two biotransformation products, EA-cysteinylglycine has not been examined for its renal or otic effects; however, EA-mercapturate is known to be devoid of diuretic properties (E. J. Cragoe, Jr., personal communication). Whether the renal and otic effects are due to EA or to one or more of its biotransformation products remains a difficult problem to resolve because of the interconvertibility of these substances.

SUMMARY

The most meaningful structure-activity relationship studies are those that take into account the multifaceted nature of the renal and extrarenal effects of the POA acid-type diuretic-saluretic agents. Throughout this review an attempt has been made to highlight the direction in which such studies should be aimed.

Structure-activity relationship studies involving the POA acids have revealed that (*a*) the sulfhydryl reactivity of EA and other POA acid-related agents is most likely not an absolute requirement for the induction of a diuretic-saluretic or an antihypertensive response, (*b*) small changes in the chemical structure of POA acids can create agents that differ drastically in their diuretic efficacy (EA and MK-196 versus ticrynafen) and uricosuric activity (EA versus ticrynafen and MK-196), and (*c*) such studies are complicated by the possibility that these agents are exerting some of their effects by a direct action on cellular processes as well as by an indirect action

that is exerted through the liberation of key prostaglandins and other regulators of cellular function. There is an intricate relationship between the renal renin-angiotensin, prostaglandin, and kinin systems, and the influence of the various POA acid-related agents on these systems can no longer be ignored. Unfortunately, the effect of the POA acids on the kinin system remains totally unexplored. Finally, structure-activity relationship studies should attempt to relate the structure and actions of one POA acid with the structure and actions of other POA acids as well as attempt to elucidate what the POA acid-type diuretic agents and the aminobenzoic acid-type diuretics have in common, mechanistically, to evoke similar beneficial and detrimental actions.

ACKNOWLEDGMENTS

Portions of the work cited in this review were supported by USPHS grant GM 26775.

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