Michaelis–Menten Kinetic Parameters of Renal Tubular Secretion of *para*-Alkylated Mandelic Acids and Their Use in Revealing Possible Chemical Characteristics around Receptor Site of Secretion Carrier in Rats

JANARDAN B. NAGWEKAR▲, PRAVIN M. PATEL, and MOIZ A. KHAMBATI

Abstract □ From the determination of the Michaelis–Menten kinetic parameters (V_m and K_m) of renal tubular secretion of D-(−)-*p*-*n*-propylmandelic acid (V) and D-(−)-*p*-isopropylmandelic acid (VI) in rats, it is shown that these compounds involve the same renal tubular carrier for their secretion but the affinity of V for the carrier is approximately twice that of VI. By considering these parameters as well as the structural variations of V and VI, together with those of other homologs of D-(−)-mandelic acid (I) studied previously, inferences have been drawn regarding the chemical nature around the possible cationic receptor site of the carrier for renal tubular secretion of organic anions in rats. The utilization of the model compounds of a homologous series of I in studying the influence of alkyl groups present in the organic anions on the pharmacokinetic parameters, particularly the volume of distribution, is discussed.

Keyphrases □ Renal tubular secretion—Michaelis–Menten kinetic parameters, *para*-alkylated mandelic acids used to determine chemical nature of secretion carrier receptor site environment, rats □ Michaelis–Menten kinetic parameters—renal tubular secretion, *para*-alkylated mandelic acids used to study chemical environment of cationic receptor sites of the carrier, influence of alkyl groups, volume of distribution, rats □ Receptor sites, renal tubular secretion carrier—Michaelis–Menten kinetic parameters, *para*-alkylated mandelic acids, influence of alkyl groups, rats □ Secretion, renal tubular, rats—*para*-alkylated mandelic acids used to study chemical environment of carrier receptor site, Michaelis–Menten kinetic parameters, influence of alkyl groups, volume of distribution □ Pharmacokinetic parameters, Michaelis–Menten—renal tubular secretion of *para*-alkylated mandelic acids, use in revealing possible chemical characteristics around the receptor site of secretion carrier, rats

The Michaelis–Menten kinetic parameters (V_m and K_m) of renal tubular secretion determined in rats for D-(−)-mandelic acid (I), tropic acid (II), D(−)-*p*-methylmandelic acid (III), and D-(−)-*p*-ethylmandelic acid (IV) were reported (1, 2) recently. The analysis of these parameters indicated that these compounds are secreted in rats by involving a common carrier and that the affinity of III for the carrier is similar to that of I but that the affinity of IV or II is almost twice that of I. The data obtained in these studies in terms of V_m and K_m were further utilized to distinguish certain chemical structural features of a possible renal tubular carrier responsible for the secretion of these compounds.

Another interesting inference drawn from previous studies (1, 2) in rats was that the apparent volume of distribution (V_d) of the *para*-alkylated mandelic acids (III and IV) is approximately half of that of I, the parent compound.

Since these compounds are neither bound to plasma proteins, metabolized, nor reabsorbed from the renal tubules, in addition to being recovered completely in the urine of rats in the unchanged form, it became possible to determine the effect of introducing alkyl groups in the *para*-position of I on the V_d, initial glomerular filtration rates, and renal tubular secretion rates of these compounds in rats. Noticing such dramatic effects of *para*-alkylation of I on the pharmacokinetic parameters of the compounds, it was considered of great interest to study in rats the effect on these parameters of substituting propyl ($CH_3CH_2CH_2$—) and isopropyl [—$CH(CH_3)_2$] groups in the *para*-position of I.

Therefore, the purpose of the present study was to determine the relative magnitudes of the V_d and the Michaelis–Menten kinetic parameters of renal tubular secretion of D-(−)-*p*-*n*-propylmandelic acid (V) and D-(−)-*p*-isopropylmandelic acid (VI) from the urinary excretion kinetic studies in rats. These data will also be utilized in ascertaining certain inferences drawn from previous studies regarding the topography of the renal tubular secretion carrier receptor around its cationic site(s) which is (are) considered to form primary sites of attachment of the compounds used in the study.

EXPERIMENTAL

Materials—The following were used: D-(−)-mandelic acid, m.p. 132–133°, $[\alpha]_D^{25}$ −154°; D-(−)-*p*-*n*-propylmandelic acid, m.p. 124–125°, $[\alpha]_D^{25}$ −142°, obtained by resolving from the racemic acid synthesized by the method of Reibsomer *et al.* (3); D-(−)-*p*-isopropylmandelic acid, m.p. 159–160°, $[\alpha]_D^{25}$ −129°, obtained by resolving from the racemic acid synthesized by adapting the method of Carson *et al.* (4) for the synthesis of I; and DL-tropic acid, m.p. 117–120°.

Apparatus and Analytical Procedure—The equipment and the GC procedure used for quantitative determination of I and its homologs appearing in the urine of rats were the same as described previously (1). The procedures for specific rotation determination, pH measurements, dissociation constant determination, partition coefficient determination, protein binding determination, and absolute configuration determination of the compounds were also described previously (1). The absolute configuration of the compounds found was D.

Methodology—The procedure followed for preparing the rats and for urine collection following intravenous administration of the compounds was the same as described previously (1). Approximately 65 Sprague–Dawley male rats, weighing 170–220 g., were used repeatedly. Of the 65 rats, 30 were used in the urinary excretion studies involving VI and the rest were used in the urinary excretion studies involving V. The aqueous solutions of sodium salt of each compound used for intravenous administration were made isotonic, and the volumes of intravenous injections varied from 1 to 2 ml. The urinary excretion kinetics of V were studied over the dosage range of 72–258 μmoles/kg. (3–10 mg./rat) and that of VI were studied over the dosage of 129–387 μmoles/kg. (5–15 mg./rat). The reason for using lower doses of V is explained later.

To determine the initial glomerular filtration rates of V at the dosage levels of 72–132 μmoles/kg., the appropriate dose of the substrate compound (V) and the 3012-μmoles/kg. dose of II as an inhibitor, both as their sodium salts contained in 2 ml., were injected intravenously to the rat.

Table I—Overall Urinary Recovery of the Compounds in the Unchanged Form

Compound	Number of Rat Studied	Dose per Rat, mg.	Recovery, mg.
DL-V	3	10	6.1–6.6
DL-VI	3	10	6.5–6.8
V	3	10	9.9–10.0
VI	6	5	4.6–4.9

To determine the nature of inhibition of the renal tubular secretion of VI by II, the urinary excretion kinetic studies of VI at the intravenous dosage levels of 206, 257, and 387 μmoles/kg. in the presence of a 1355-μmoles/kg. dose of II were carried out. Both VI and II were administered as their sodium salts and were contained in the same intravenous solution.

In studies where the apparent initial glomerular filtration rates of VI and I were simultaneously determined in the same rat in the presence of 3012 μmoles/kg. of II as the inhibitor of secretion of these compounds, the equimolar doses of VI and I were used. Three different doses of these compounds were employed: 180, 258, and 387 μmoles/kg.

RESULTS AND DISCUSSION

Selection of Optical Isomers of Compounds—The selection of V and VI for the present study was based on the following ideal properties possessed by the compounds.

1. From the overall urinary recovery (Table I) of the administered intravenous doses of racemic *p*-propylmandelic acid, racemic *p*-isopropylmandelic acid, V, and VI, it becomes clear that only with the levorotatory isomers of the compounds is virtually 100% of the given doses recovered in the unchanged form. The poor recovery of the compounds (unchanged) following administration of the racemic form was due to metabolism of the dextrorotatory form of the compounds, as found previously for other homologs of I (1, 2). In view of the objectives of the study, quantitative recovery of V and VI was considered to be ideal.

2. The pKa value reported for VI is 3.64 (5) and that determined for V is 3.7. Therefore, these compounds are expected to remain in the ionized form in the biological fluids.

3. The partition coefficients ($C_{organic}/C_{buffer}$) determined for these compounds according to the procedure previously described (2) were zero, which lead to the reasonable assumption that the reabsorption of the compounds in the renal tubules is negligible, if any.

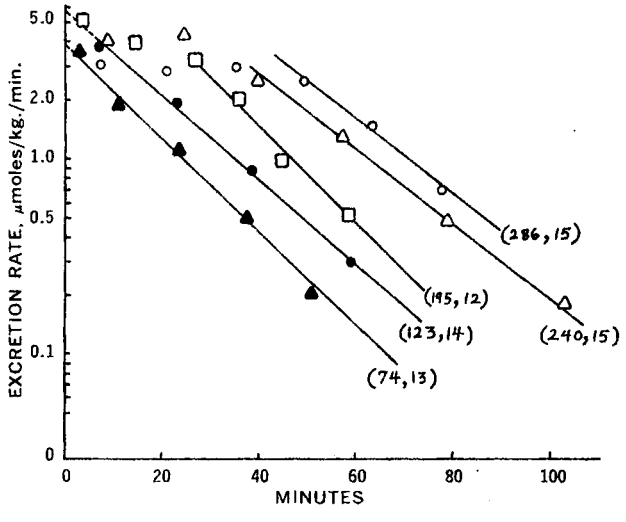

Figure 1—*Plots of log urinary excretion rate* versus *time for V following intravenous administration of various doses to rats. (The numbers in parentheses refer to intravenous dose in micromoles per kilogram and the biological half-life of the compound, respectively. At doses of 240 and 286 μmoles/kg., the biological half-life refers to the data obtained after 30 min., as explained in the text.)*

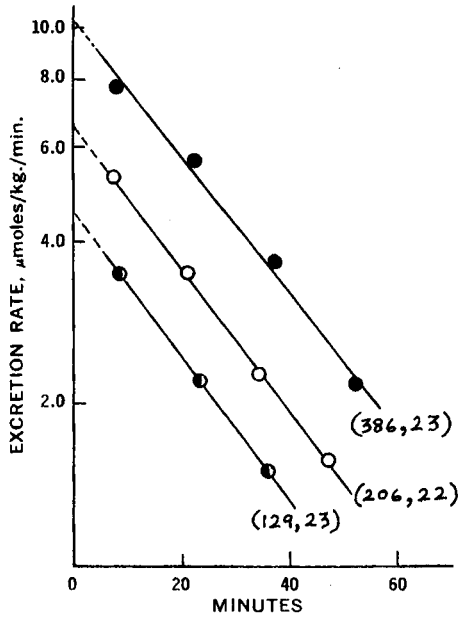

Figure 2—*Apparent first-order urinary excretion of VI following intravenous administration of various intravenous doses to rats. (The numbers in parentheses refer to the intravenous dose in micromoles per kilogram and the biological half-life of the compound, respectively.)*

4. From the equilibrium dialysis studies, it was noticed that at the equilibrium concentrations of 0.152–0.255 mg./4 ml. of V in the plasma, about 3.7–5.2% of the compound was bound. For practical purposes, this extent of binding was considered negligible. The binding of VI to plasma proteins was found to be negligible.

Urinary Excretion Kinetics of V and VI—As can be seen from the data presented in Fig. 1, two different patterns of urinary excretion of V in rats become evident. At the intravenous doses of 72–143 μmoles/kg. studied, the excretion of V occurred by an apparent first-order process and the kinetic model for the compound was indeed describable as the one-compartment open model. Since the administered dose of the compound is recovered entirely in the urine in the unchanged form, the urinary excretion data were treated according to the following equation:

$$\log \Delta Ae/\Delta t = \log kA_0 - kt/2.303 \qquad \text{(Eq. 1)}$$

where $\Delta Ae/\Delta t$ is the rate of excretion of the compound at time t, which is the midpoint of the urine collection interval; A_0 is the amount of the compound in the body at zero time, k is the apparent first-order rate constant for excretion of the compound, and kA_0 is the apparent initial excretion rate of the compound at a given intravenous dose. The rate constant k was obtained from the slope ($-k/2.303$), and the initial excretion rate was obtained from the intercept of the straight line obtained upon plotting log $\Delta Ae/\Delta t$ *versus* t. The straight line in each case was obtained by the method of least squares. The rate constant k is designated as the rate constant of urinary excretion because the administered doses of the compound are recovered entirely in the urine in the unchanged form (1). The biological half-life of Compound V was calculated from $0.693/k$. The average biological half-life of the compound determined from 23 studies over the intravenous dosage range of 72–143 μmoles/kg. was 13 min.

However, when the studies were carried out at the intravenous dosage levels of 195–294 μmoles/kg., the excretion of Compound V in almost all of the 11 studies was found to take place by an apparent first-order process (with the biological half-life of about 12 min.), but generally after 30 min. During the initial 30 min., the excretion rate of the compound declined so gradually that the rates appeared to be almost constant (Fig. 1). Although it may be expected that, since V is secreted by the renal tubules of rats as shown later, the biological half-life of the compound would tend to increase with the increase in the dose due to increasing saturation of the secretory process, such an extremely gradual decline in the excretion rate noted during the initial period cannot be accounted for

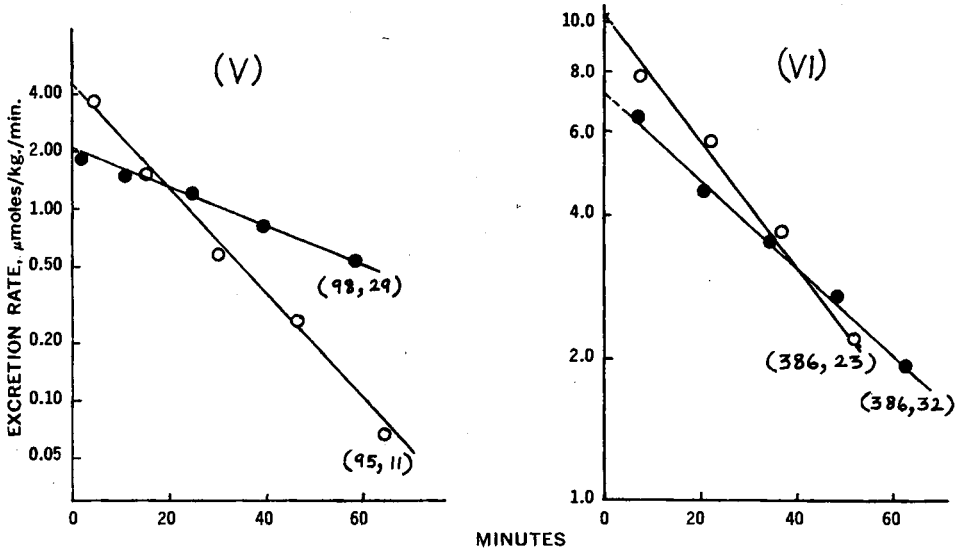

Figure 3—*Apparent first-order urinary excretion of V and VI in the absence* (○) *and in the presence* (●) *of a simultaneously administered 3020-μmoles/kg. i.v. dose of II. (The numbers in parentheses refer to the intravenous dose in micromoles per kilogram of the respective compound and its biological half-life, respectively.)*

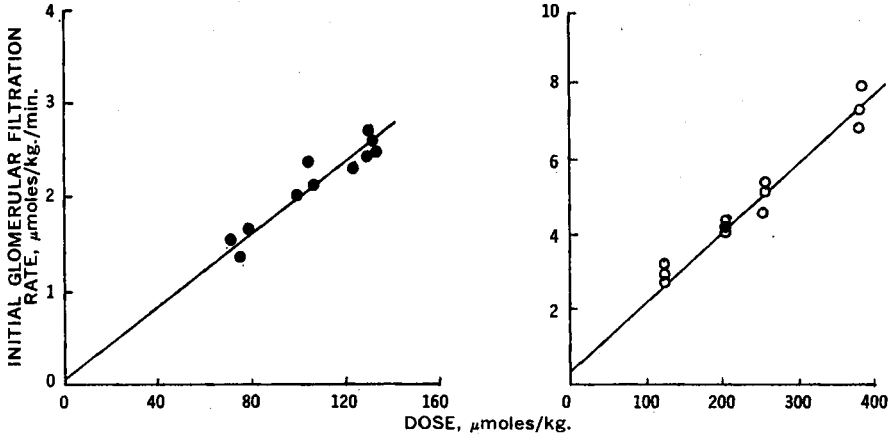

Figure 4—*Relationship between the apparent initial glomerular filtration rates and the intravenous doses for V* (●) *and VI* (○).

on this basis. The reason for this belief is that even IV, which has almost the same V_d and affinity for the renal tubular secretion carrier in rats, did not show such a saturation effect, as evidenced by the constancy of its biological half-life even up to a 885-μmoles/kg. i.v. dose (2). The reason for this phenomenon has been investigated and will be reported separately.

The facts that VI is excreted by an apparent first-order process and that its excretion is describable by the one-compartment open model become evident from the data presented in Fig. 2. The average biological half-life determined for the compound is 23 min.

Determination of Apparent Initial Glomerular Filtration Rates and Apparent Renal Tubular Secretion Rates—In previous studies (2), II was shown to inhibit competitively the renal tubular secretion of the homologs of I in rats. Therefore, II was used to inhibit the tubular secretion of 129–387-μmoles/kg. doses of VI or 72–132-μmoles/kg. doses of V. The preliminary studies indicated that the apparent initial excretion rates obtained for the compounds at these doses were the same in the presence of a 3012- or a 4500-μmoles/kg. i.v. dose of II, indicating that the dose of 3012 μmoles/kg. of II was adequate to inhibit the secretion of the compounds. Therefore, in each study, a 3012-μmoles/kg. dose of II (inhibitor), along with an appropriate dose of each compound, was administered to the rat intravenously. Both the substrate compound and the inhibitor were administered as their sodium salts contained in a 2.5-ml. isotonic solution. The overall urinary recovery of the doses of the substrate compounds in these studies was found to be quantitative and, as evidenced from the typical studies presented in Fig. 3, the excretion of the substrate compounds in the presence of II occurred by an apparent first-order process. The apparent initial excretion rates obtained in these studies were plotted against the corresponding doses of the compounds (Fig. 4). As shown in this figure, the least-squares straight lines obtained for the data almost passed through the origin, indicating that the apparent initial excretion rates determined in these studies represent the apparent initial glomerular filtration rates of the compounds at the corresponding intravenous doses.

The apparent initial secretion rates were computed by subtracting the initial glomerular filtration rates from the apparent initial excretion rates at the corresponding intravenous doses of each compound. The glomerular filtration rates used in the computation of the apparent initial secretion rates were, however, those obtained from the representative plots (Fig. 4) corrected to pass through the origin.

The composite plots of apparent initial excretion rates, initial glomerular filtration rates, and initial secretion rates *versus* intravenous doses of the compounds are shown in Figs. 5 and 6.

Determination of Michaelis–Menten Kinetic Parameters for Renal Tubular Secretion—As previously demonstrated (1, 2), the data regarding the initial secretion rates (V) and the intravenous doses (D) were treated according to the Lineweaver–Burk equation:

$$1/V = K_m/V_mD + 1/V_m \qquad \text{(Eq. 2)}$$

and the values of the apparent maximum initial secretion rate (V_m) and the intravenous dose (K_m) required to produce 1/2 V_m were calculated from the straight-line (least-squares) plot obtained for each compound upon plotting $1/V$ *versus* $1/D$ (Fig. 7). The respective

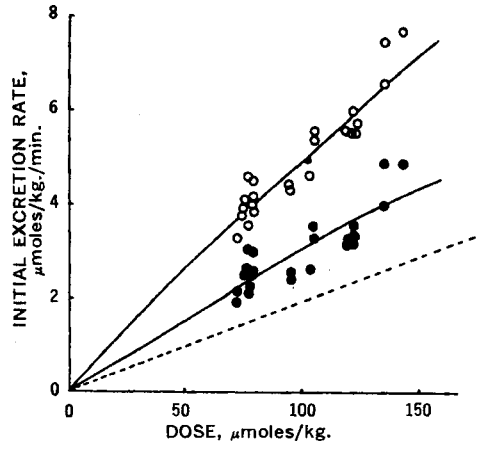

Figure 5—*Apparent initial excretion rates* (○), *initial glomerular filtration rates* (- - -), *and initial secretion rates* (●) *obtained for V following the intravenous administration of appropriate doses to rats.*

values of V_m for these compounds were calculated from the intercepts on the y-axis, and those of K_m were calculated from the slopes of the straight lines. The values of V_m and K_m are listed in Table II, along with those for I, II, III, and IV for comparison.

Although no claim can be made that the values of V_m and K_m (Table II) obtained from four different studies are precise enough to allow a distinction with respect to the precise affinity of the various compounds for the common carrier, it is reasonable to conclude that all of these compounds are secreted by the same renal tubular secretion carrier. As additional support to this conclusion, studies were carried out to show that II competitively inhibits the secretion of VI in rats. From the apparent initial excretion rates determined for VI (206–287 μmoles/kg.) in the presence of simultaneously administered 1355-μmoles/kg. i.v. doses of II, the apparent initial secretion rates were determined by subtraction from their apparent initial glomerular filtration rates at the corresponding doses. The plot of $1/V$ *versus* $1/D$ obtained for the studies is shown in Fig. 7. The apparent common y-intercept noted in this figure indicates that II competitively inhibits the secretion of VI.

Simultaneous Determination of Apparent Initial Glomerular Filtration Rates of VI and I in the Same Rats—In the present studies, it is noted that at the equimolar doses the apparent glomerular filtration rates of VI are similar (but not identical) to that of V, which in turn appear to be similar to that of III or IV reported previously (2). Randinitis *et al.* (1) showed that at the equimolar

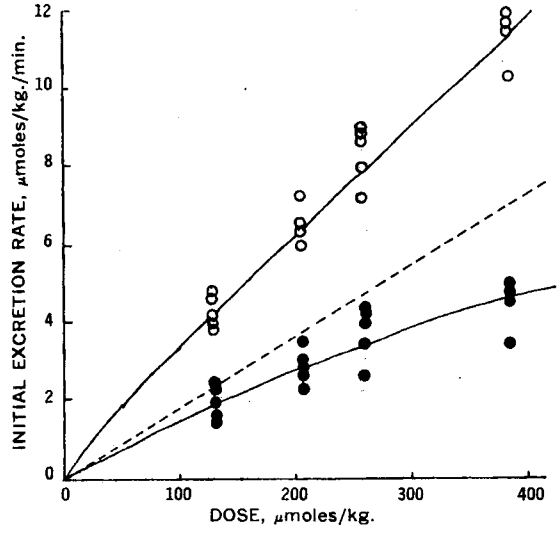

Figure 6—*Apparent initial excretion rates* (○), *initial glomerular filtration rates* (- - -), *and initial secretion rates* (●) *obtained for VI following intravenous administration of appropriate doses to rats.*

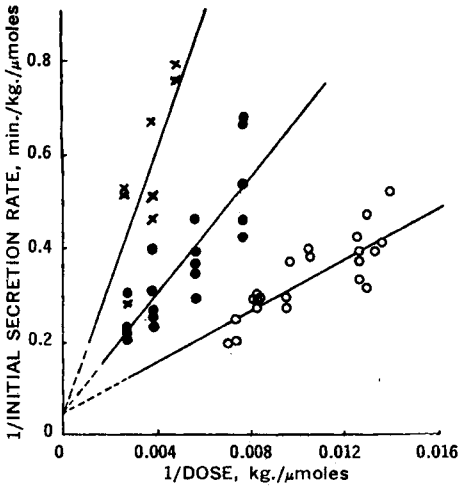

Figure 7—*Lineweaver–Burk plots of the reciprocal of intravenous doses* versus *the reciprocal of apparent initial secretion rates for V* (○) *and VI* (●) *in the absence of II and for VI* (×) *in the presence of a simultaneously administered 1355-μmoles/kg. i.v. dose of II (inhibitor).*

doses the initial glomerular filtration rates of I and its homologs obtained by introducing methylene groups in the side chain (*i.e.*, II, 4-hydroxy-4-phenylbutanoic acid, phenyllactic acid, and benzyllactic acid) are also similar. The inference (2) that the V_d in rats for III or IV is approximately half of that for I or II was based on the comparison of the apparent initial glomerular filtration rates reported in these two separate studies.

To substantiate further that the V_d of the homologs of I obtained by introducing methylene groups in the side chain is almost twice the V_d of the homologs of I obtained by *para*-alkylation, the initial glomerular filtration rates of VI and I (each representing the respective group of the homologs) were determined in the same rats by simultaneously administering the appropriate equimolar intravenous doses (180, 258, or 387 μmoles/kg.) in the presence of a 3012-μmoles/kg. dose of II. Typical data are presented in Fig. 8, and the data regarding the apparent initial glomerular filtration rates against the doses of the compounds are presented in Fig. 9. It is evident from these data that the V_d of I is almost 1.7 times greater than the V_d of VI.

Possible Interpretation of Michaelis–Menten Kinetic Parameter Data to Elucidate the Chemical Features around the Receptor Site(s) of the Renal Tubular Secretion Carrier—Since I and its various homologs (pKa 3.4–3.7) exist in the blood (pH 7.4) in ionized form, it has been tacitly assumed that the primary interaction between these compounds and the renal tubular secretion carrier present in the tubular membrane occurs through electrostatic forces. The anions of these acids are supposed to interact with the cationic receptor site(s) of the carrier.

From examination of the data in Table II, it is noted that, among the *para*-alkylated homologs of I, the affinity of V is about twice that of VI, III, and I. Although a large hydrophobic group is present, namely the isopropyl group in a VI molecule, the affinity of the molecule for the secretion "carrier" is not different from that of I or III. Therefore, it may be instinctively deduced that, among the

Table II—Michaelis–Menten Kinetic Parameters of Renal Tubular Secretion of I and Its Homologs in Rats

Compound	Apparent V_m, μmoles/kg./min.	Apparent K_m, μmoles/kg.
I[a]	18.5	11,100
II[a]	20.8	5,200
III[b]	21.6	10,200
IV[b]	18.5	4,900
V	19.6	5,640
VI	17.7	11,200

[a] Values for these compounds were obtained from *Reference 1*. [b] Values for these compounds were obtained from *Reference 2*.

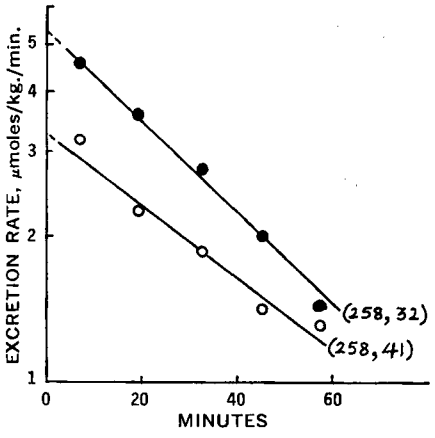

Figure 8—*Apparent first-order urinary excretion of VI (●) and I (○) in the presence of a simultaneously administered 3012-μmoles/kg. i.v. dose of II (inhibitor). (The numbers in parentheses refer to the intravenous dose in micromoles per kilogram of the respective compound and its biological half-life, respectively.)*

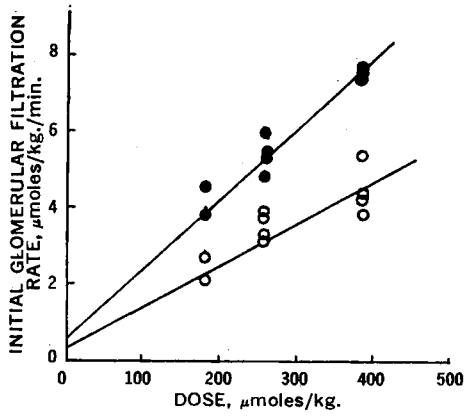

Figure 9—*Comparison of the apparent initial glomerular filtration rates observed for VI (●) and I (○) in rats.*

para-alkylated homologs of I, the length of the hydrophobic group attached in the *para*-position in the phenyl group of I determines the extent of hydrophobic interaction of the compounds with the carrier. But it is further noted that the affinity of V for the carrier is almost the same as that of IV, indicating that the length of the alkyl group in the *para*-position of the homolog molecule alone does not determine the affinity of the molecule for the carrier.

From previous studies (1) in rats involving I and its homologs [obtained by introducing methylene group(s) in the side chain of I], the possible chemical structural characteristics distinguished were that the hydrophobic region around the cationic site of the carrier molecule probably does not extend uninterruptedly beyond the hydrophobic region represented by two carbon atoms (possibly two methylene groups) and, following such hydrophobic region, there is a polar group present. This interpretation to envision the chemical nature of the receptor of the carrier was also supported by the analysis of Michaelis–Menten kinetic parameter data obtained for the *para*-alkylated homologs of I, namely, III and IV (2). Since the *para*-substitution of an ethyl group, but not that of methyl (2), resulted in increasing the affinity of the resulting molecule, as evidenced by the K_m values, it was further suggested (2) that the increased affinity of IV for the carrier is due to the additional interaction of its end methyl group with the hydrophobic portion that should be present next to the polar group of the carrier molecule.

Although it is not known exactly what component of the renal tubular membrane constitutes the carrier for the organic anions, proteins and/or phospholipid(s) have been suggested as the carrier (6, 7). Without being specific about the exact chemical entity of the membrane that is responsible for the transmembrane transport, it seems reasonable to assume that the chemical component of the membrane that serves as the carrier for the organic anions should have cationic groups. Therefore, it is believed that, because of their suitable physicochemical properties, I and its homologs used in past and present studies can serve as probes to reveal the chemical nature of the renal tubular carrier around its cationic receptor site(s). In the present study, the *para*-substitution of the *n*-propyl group in I did not result in further increase in the affinity of the resulting molecule (*i.e.*, V) for the carrier than that of IV. Owing to the additional methylene group, if the affinity of V for the carrier were to be higher than that of IV, hydrophobic interaction was expected to occur between the additional methylene (or methyl) group of V and the complementary hydrophobic group of the receptor site of the carrier. The lack of such increase in the affinity of V for the carrier may be interpreted to suggest that the end methyl group of V comes in contact not with a nonpolar group but possibly with a polar group. Thus, this study serves to distinguish a further structural characteristic of the carrier by indicating that the hydrophobic group suggested to be present next to the polar group of the carrier from the studies of (IV) (2) is probably represented by one carbon atom (possibly a methylene group), and next to this group there is present probably a polar group.

Pharmacokinetic Significance—To induce a biologial effect, it is well recognized that the drug should be present in effective concentration at the site of action and interact with the receptors in some appropriate manner. It is further recognized that there is a relationship between the physicochemical properties of the drug and its biological activity (8), because the attainment of effective concentration of the drug at the receptors is a function of several rate processes the drug is simultaneously subjected to in the body. These rate processes include the rate of access of the drug to, and the rate of disappearance of the drug from, its site of action, the rate of metabolism, the rate of glomerular filtration and renal tubular secretion, and the extent of binding of drug to plasma proteins. These processes depend on the physicochemical properties of the drug and, therefore, often rather complicated structure–activity relations are found even for compounds of a homologous series (8). It is generally believed that the introduction of hydrophobic groups in a given drug molecule tends to increase its lipid solubility, giving rise to its increased permeability into the tissue. Unfortunately, however, it does not become possible in the case of a majority of drugs to evaluate this specific effect of a hydrophobic group on the tissue permeability of the drug, since the presence of the same hydrophobic group in the drug molecule may simultaneously result in changes in its rate of metabolism, rate of excretion, and/or extent of binding to plasma proteins and thus complicate such evaluation of a single pharmacokinetic factor.

However, it is reasonable to expect that the fewer above-mentioned pharmacokinetic processes the drug is subjected to in the body the greater is the opportunity for success in correlating the effect of systematic introduction of the hydrophobic group in a drug molecule on a given pharmacokinetic process or parameter. It is in this context that I and its homologs (1, 2) are considered as the model compounds because these compounds (organic anions) are neither metabolized, bound to plasma proteins, nor reabsorbed in the renal tubules of rats. Consequently, it is possible to determine the effect of alkyl groups on the V_d, glomerular filtration rates, and renal tubular secretion rates. It is not meant to imply that the introduction of methyl or methylene groups in an organic molecule will always bring about such changes in the pharmacokinetic parameters, regardless of the nature of the organic compound. Such generalization cannot be made from the limited studies discussed here. Nevertheless, the striking effects observed of *para*-alkylation of I on the V_d, glomerular filtration rates, and tubular secretion rates are indicative of the possibility that further studies with suitable compounds would eventually provide definite insight as to the effects of alkyl substitution in the organic anions on their pharmacokinetic parameters.

The introduction of hydrophobic groups in a drug molecule is generally believed to tend to increase the lipid (tissue) solubility of the compound, consequently resulting in its increase in the V_d. But the effect of introducing alkyl groups in the *para*-position of the organic anions in the present homologous series was found to be just the opposite. The V_d of the *para*-alkylated homologs of I decreased while that of II remained practically unchanged (1), as compared to the V_d of the parent compound, I. This illustrates the influence of the position of the alkyl group in the compound on its V_d in the body.

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▲ To whom inquiries should be directed.

Chronic Isoproterenol Treatment of Neonatal Rats

GERDA I. KLINGMAN▲ and GENIE McKAY

Abstract □ The chronic subcutaneous administration of isoproterenol to neonatal and young male and female rats resulted in statistically significant reductions of the norepinephrine concentrations of the heart and submaxillary and parotid glands and increased concentrations in the stellate ganglion. However, the total norepinephrine content of these tissues was not affected. The epinephrine levels of these tissues were not significantly different from controls except for an increased total content in the submaxillary glands. Chronic treatment did not alter the norepinephrine levels of the brain, superior cervical ganglion, adrenal glands, vas deferens, uterus, spleen, kidneys, lungs, and small intestine. Growth of neonatal rats was retarded. The wet weights of the submaxillary and parotid glands were significantly greater, while the spleen, uterus, adrenal glands, and stellate ganglion weighed less in isoproterenol-treated than in control rats. The wet weights of other peripheral tissues were not affected. The ratios of the tissue weight to the body weight were significantly increased for the heart and submaxillary and parotid glands and decreased for the uterus. The first dose of isoproterenol produced hypothermia in mature rats but hyperthermia in newborn rats. Sparse hair growth in neonatal rats and hair loss in weanling rats were also observed during chronic treatment.

Keyphrases □ Isoproterenol, chronic administration—effects on tissue catecholamine levels, tissue weights, and rectal temperature in rats □ Catecholamine tissue levels—effect of chronic isoproterenol administation, rats □ Tissue weight and catecholamine levels—effect of chronic isoproterenol administration, rats

The ability of isoproterenol to produce hypertrophy and hyperplasia of the submaxillary and parotid glands of rats has been studied by various investigators (1–5). In addition, isoproterenol treatment resulted in infarct-like lesions and fatty degeneration of the heart (6, 7). Effects in other species have also been reported, *e.g.*, cats (8), hamsters (9), guinea pigs (10), and mice (11, 12). Growth stunting, premature opening of the eyes, and an increase of the ratio of heart weight to body weight have been reported following the chronic administration of isoproterenol to neonatal rats (5). Since acute and short-term treatment resulted in increased organ weights and derangements of catecholamine levels (13–15) and storage (15), and chronic isoproterenol treatment produced growth stunting and changes in organ weights in neonatal rats (5), the effects of chronic isoproterenol treatment on the tissue catecholamine levels, tissue weights, and rectal temperature were studied in neonatal rats and the results are reported in this paper.

METHODS

Animals—Two days after birth, Sprague–Dawley male and female rats from three litters were subcutaneously dosed with 0.85% sodium chloride solution (controls) or 5 mg./kg. isoproterenol twice a day for 6 days and thereafter with 15 mg./kg. twice a day for up to 43 days. Animals of two other litters received 10 mg./kg. subcutaneously twice a day for 7 days, the first dose being given on the 6th day after birth. At the end of the 1st week of drug administration, nearly 50% of the animals of this group had died and the dose was reduced to 10 mg./kg. once a day.

To obtain additional information on the hyperthermia, the frequently observed lethargy, and the sparsity of hair growth (or hair loss), animals of three other litters were treated chronically by three different dose regimens: 20 mg./kg. once a day and 5 mg./kg. twice a day for 5 days (treatment was started on the 6th day after birth) and 15 mg./kg. twice a day for 30 days (treatment was started on the 25th day after birth).

Catecholamine Analysis—For the tissue catecholamine measurements, the treated animals and their littermate controls were sacrificed by exsanguination under sodium pentobarbital anesthesia after 30–43 days of treatment. The heart, submaxillary glands, parotid glands, spleen, kidneys, lungs, small intestine, vas deferens, uterus, adrenal glands, whole brain (or brainstem[1]), superior cervical ganglia, and stellate ganglia were dissected in the cold and weighed[2]. Tissues were analyzed immediately or frozen and analyzed within 3 days after dissection. Norepinephrine and epinephrine were extracted with 1-butanol as previously described (16) and measured spectrophotofluorometrically by a modification (17, 18) of the ferricyanide oxidation method of von Euler and Floding (19).

[1] In this report the brainstem is defined as the medulla, the pons, the midbrain, and the diencephalon.

[2] On a Mettler balance or a Cahn electrobalance.