Marcaine, a Selective Inhibitor of Eucaryotic Aminoacylation†

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ABSTRACT: The effects of marcaine, a myotoxic drug, on the aminoacylation of transfer ribonucleic acid (tRNA) have been studied. The drug is a potent inhibitor of the acylation of rat liver tRNA with leucine and isoleucine but is only mildly inhibitory (or not inhibitory) to acylation with a number of other amino acids which were tested. Further, marcaine inhibited aminoacylation in cell-free systems using components from several mammalian tissues, including muscle, from yeast, and from wheat germ. No effect of the drug was observed in aminoacylation systems from several bacterial species which were tested. The drug inhibits acylation with leucine and isoleucine competitively but exhibited noncompetitive kinetics when the concentrations of adenosine 5′-triphosphate (ATP) and tRNA were varied. Marcaine was also a competitor of leucine in the ATP–pyrophosphate exchange reaction. Two structural analogues of marcaine, carbocaine and xylocaine, also inhibited acylation of rat liver tRNA with leucine but in a noncompetitive fashion. On a molar basis, marcaine appears to be the most effective inhibitor of the three drugs tested.

Marcaine (bupivicaine, *dl*-1-butylpipecoloxylidide, Figure 1) is a myotoxic drug which has been used clinically as a local anesthetic (TeLuvio, 1963). It has been shown to produce rapid degeneration of skeletal muscle fibers and has been used recently in studies of skeletal muscle regeneration (Hall-Craggs, 1974; Carlson, 1976). Treatment of certain mammalian skeletal muscles with marcaine, after removal of the muscle from the experimental animal but prior to autogenous free grafting, results in a rapid degeneration of the transplanted muscle fibers followed by a regenerative phase in which the weight and contractile properties of the transplants are restored to levels which are 50–90% of those for control muscles (Carlson, 1976; Carlson & Gutmann, 1976). Although some biochemical studies on marcaine-treated skeletal muscle have been performed (Wagner et al., 1976, 1977), little information is available on the effects of the drug at the molecular level. In a recent report from this laboratory, it was demonstrated that marcaine, while without effect on RNA synthesis in skeletal muscle, was a potent inhibitor of protein synthesis, both in intact muscle and in cell-free systems prepared from that muscle (Johnson & Jones, 1978). Further, it was shown that while marcaine slightly inhibited the elongation of polypeptide chains in a cell-free system derived from rat skeletal muscle, it was a stronger inhibitor of the aminoacylation of tRNA. This inhibitory effect appeared to be confined to aminoacylation systems of mammalian origin (Johnson & Jones, 1978).

This interesting effect of marcaine on the acylation of tRNA certainly warranted further investigation. To that end, the action of the drug has been carefully studied in terms of its amino acid specificity, selectivity for eucaryotic aminoacylation, and inhibition kinetics. In addition, the inhibitory activity of marcaine has been compared with that of two other drugs of similar structure. The results of these studies are reported below.

Materials and Methods

Materials. Adult male Sprague–Dawley rats were used as the source of liver and skeletal muscle in the studies described below. Marcaine, carbocaine, and xylocaine were generously supplied by Dr. F. C. Nachod (Sterling-Winthrop Research Institute, Rensselaer, NY) and were prepared as 5 or 10% (w/v) solutions in 45% ethanol. Niblach's raw wheat germ was obtained from a local health food store and commercial baker's yeast from a local bakery. Frozen cells of *Escherichia coli* B were from Miles, *Pseudomonas fluorescens* cells were from Vega-Fox, and *Streptomyces antibioticus* cells were grown as previously described (Jones, 1976). *E. coli* tRNA was purchased from Schwarz/Mann. The following radioisotopes were obtained from Amersham: [^{3}H]leucine (50 Ci/mmol); [^{3}H]isoleucine (17 Ci/mmol); [^{3}H]lysine (97.5 Ci/mmol); [^{3}H]methionine (6 Ci/mmol); [^{3}H]serine (17 Ci/mmol); [^{3}H]glycine (33 Ci/mmol); and [^{3}H]phenylalanine (17 Ci/mmol). [^{3}H]Valine (1.3 Ci/mmol), [^{3}H]threonine (2.38 Ci/mmol), [^{3}H]glutamic acid (49 Ci/mmol), and sodium [^{32}P]pyrophosphate (95 mCi/mmol) were from New England Nuclear.

Preparation of Crude tRNA and Aminoacyl-tRNA Synthetase. Total tRNA was prepared from rat liver, skeletal muscle, murine myeloma MOPC-41, wheat germ, baker's yeast, *E. coli*, *P. fluorescens*, and *S. antibioticus* by previously published methods (Bridges & Jones, 1973; Jones, 1975). Crude aminoacyl-tRNA synthetase was prepared as described previously (Muench & Berg, 1966; Bridges & Jones, 1973). Briefly, the procedures involved homogenization or sonication of the appropriate tissue or organism, high-speed centrifugation, passage of the high-speed supernatant through DEAE-cellulose to remove RNA, and dialysis of the DEAE-cellulose treated enzyme against 50% glycerol in an appropriate buffer.

Partial Purification of Rat Liver Leucyl-tRNA Synthetase. A partially purified leucyl-tRNA synthetase preparation was used for the kinetic studies to be reported below. Rat liver was chosen as the source of this enzyme because of the availability of large quantities of this tissue. Step 1: 12 grams of rat liver was homogenized in a Teflon–glass homogenizer in 25 mL of medium A (50 mM Tris-HCl, 25 mM KCl, 5 mM $MgCl_2$, and 5 mM 2-mercaptoethanol, pH 7.6) containing 20% glycerol. The homogenate was centrifuged for 10 min at 13 000*g* and the resulting supernatant centrifuged again under the same conditions. Step 2: the final supernatant was made 0.2 M in KCl and centrifuged for 2 h at 200000*g*. Step 3: the 200000*g* supernatant was diluted with medium A to a protein concentration of 20 mg/mL and brought to 35% saturation with solid ammonium sulfate added with constant stirring. After being stirred for 15 min at 0 °C, the suspension was centrifuged for 10 min at 20000*g*. The resulting supernatant was then brought to 65% saturation with ammonium sulfate,

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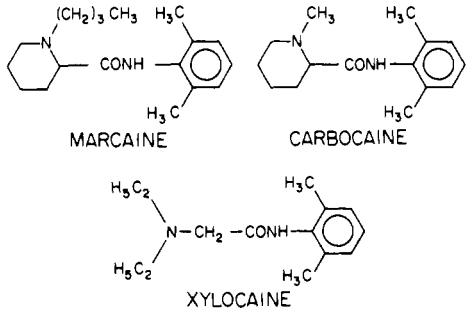

FIGURE 1: Structures of marcaine, carbocaine, and xylocaine.

and the protein precipitate was collected as described above. Step 4: the resulting precipitate was dissolved in a minimal volume of medium A containing 20% glycerol and applied to a 1.5 × 95 cm column of Bio-Gel A.5m eluted with the same buffer. Fractions of 1.5 mL were collected at a flow rate of 15 mL/h. Step 5: enzyme-containing fractions from the Bio-Gel column were pooled and applied to a 2 × 11 cm column of DEAE-cellulose equilibrated with medium A–20% glycerol. The column was washed with this buffer until the A_{280} of the effluent was near zero and then connected to a 200-mL linear gradient of 0.025–0.355 M KCl in medium A containing 20% glycerol. Fractions of 2 mL were collected at a flow rate of 40 mL/h. Enzyme-containing fractions were pooled and dialyzed against medium A containing 50% glycerol.

The partially purified enzyme was assayed for protease contamination by incubation of varying amounts for 60 min at 37 °C and assaying for the release of amino acids and peptides by the ninhydrin method (Spies, 1957). Inorganic pyrophosphatase assays were performed as described by Josse (1966).

Conditions for Aminoacylation of tRNA. In all assays except those performed for kinetic analysis, reaction mixtures for aminoacylation contained the following: 50 mM Tris-HCl, pH 7.6; 5 mM $MgCl_2$; 5 mM KCl; 5 mM 2-mercaptoethanol; 5 mM ATP; 4–10 A_{260} units of tRNA per mL; 0.08–2.9 mg/mL aminoacyl-tRNA synthetase protein; and the ^{3}H-labeled amino acid, 100 μCi/mL at a concentration of 2–250 μM (see legends to the tables and figures for the exact concentrations). Marcaine, carbocaine, or xylocaine was added to some reaction mixtures at the concentrations indicated in the legends to the tables and figures. Reaction mixtures (usually 100 μL) were incubated for 5–10 min at 37 °C. These and all other assays were performed in duplicate. Aminoacyl-tRNA was precipitated with trichloroacetic acid, collected on glass fiber filters, and quantitated by liquid scintillation counting. For the purposes of the leucyl-tRNA synthetase purification, one enzyme unit represents the esterification of 1 nmol of leucine in a 5-min incubation under the assay conditions described above.

Kinetic assays were performed under conditions similar to those just described, except that the concentration of one substrate was varied while the concentrations of the other two were held constant at saturating levels (5 mM ATP, 0.1 mM leucine, and 40 μM tRNA). tRNA concentration was varied between 2 and 40 μM, calculated assuming that 1 mg of tRNA is equivalent to 20 A_{260} units and that the molecular weight of the tRNA used in this study was 25 000. The percentage of the total tRNA represented by $tRNA^{Leu}$ was determined by charging 143 pmol of total tRNA with excess aminoacyl-tRNA synthetase in the presence of 100 μM leucine. Under these conditions, it was estimated that $tRNA^{Leu}$ represented 1.98% of the total tRNA population. This value was used in calculations involving the data of Figure 3A and in determining the K_m for tRNA. Leucine concentration was varied between 0.015 and 0.13 mM, and isoleucine concentration was varied between 0.00375 and 0.0625 mM. The ATP concentration was varied between 0.5 and 5 mM. Partially purified leucyl-tRNA synthetase was present at a concentration of 0.08 mg/mL, except in the studies with isoleucine when crude rat liver synthetase (2.9 mg/mL) was used. Marcaine, carbocaine, or xylocaine was present in some incubation mixtures at the concentrations indicated in the legends to the appropriate figures. Reaction mixtures were incubated for 5 min at 37 °C and processed as described above. Preliminary time course experiments showed that acylation did proceed linearly for the 5-min incubation period. A zero time incorporation value was subtracted from all incorporation data. In experiments in which the amino acid concentration varied, zero time control reaction mixtures corresponding to each amino acid concentration were prepared and the blank value was subtracted from the experimental value for the corresponding amino acid concentration. Kinetic data were plotted by the Lineweaver–Burk method and the best straight lines drawn through the data points by least-squares analysis. Kinetic parameters were determined from the least-squares equation for each line.

Since marcaine stock solutions were prepared in ethanol, it was important to determine whether the presence of ethanol alone had any effect on the acylation reactions. The greatest ethanol concentration used in any drug inhibition experiment was 4.5% (v/v). This ethanol concentration caused no more than a 25% change in the level of aminoacylation, as compared with controls lacking ethanol, for any of the 10 amino acids tested in this study. For example, 4.5% ethanol increased the level of acylation of rat liver tRNA with leucine by 12.6%. In order to control for possible effects of ethanol on the aminoacylation reaction, we added ethanol to incubation tubes lacking drug to give the same ethanol concentration present in tubes containing marcaine or other inhibitor.

Conditions for ATP–$^{32}PP_i$ Exchange. The ATP–$^{32}PP_i$ exchange reaction was performed essentially as described by Calendar & Berg (1966). Reaction mixtures (100 μL) contained the following: 100 mM Tris-HCl, pH 7.6; 10 mM potassium fluoride; 5 mM ATP; 5 mM sodium pyrophosphate (about 400 000 cpm/μmol); 5 mM $MgCl_2$; 5 mM 2-mercaptoethanol; 0.05–1.5 mM leucine; 0.5 mg/mL bovine serum albumin; and partially purified leucyl-tRNA synthetase, 0.17 mg/mL. Reaction mixtures were incubated for 15 min at 37 °C, during which period ATP–pyrophosphate exchange proceded linearly, and labeled ATP was collected as described by Calendar & Berg (1966). Marcaine was present in some experiments at 15.4 or 30.8 mM, and carbocaine (35.4 mM) or xylocaine (36.9 mM) was added in other experiments. A zero time incorporation value was subtracted from each experimental value. Kinetic data were plotted and analyzed as described above.

Miscellaneous Methods. Protein was determined with the Bio-Rad protein assay reagent.

Results

Effects of Marcaine on the Aminoacylation of Rat Liver tRNA. In a previous study, it was shown that marcaine was a potent inhibitor of the acylation of mammalian tRNA's with certain amino acids (Johnson & Jones, 1978). These results are confirmed and extended in the experiments presented in Table I. In these experiments, the effects of the drug on the acylation of tRNA with amino acids bearing side chains representing each of the major chemical groups (hydrophobic,

Table I: Effect of Marcaine on the Acylation of Rat Liver tRNA with Various Amino Acids[a]

amino acid	pmol of amino acid acylated/min	
	MAR	+MAR
Leu	0.038	0.017
Ile	0.056	0.028
Val	0.172	0.146
Lys	0.080	0.071
Thr	0.242	0.238
Met	0.104	0.107
Ser	0.042	0.063
Gly	0.042	0.045
Phe	0.060	0.064
Glu	0.038	0.040

[a] Reaction mixtures were prepared and incubated as described under Materials and Methods by using crude rat liver synthetase (2.9 mg of protein/mL). Amino acids were present at a concentration of 10 μM and 10 μCi of ^{3}H-labeled amino acid was present per 100 μL of reaction mixture. When present, the marcaine (MAR) concentration was 15.4 mM. The tRNA concentration was 5.7 A_{260}/mL.

Table II: Effects of Marcaine on the Acylation of Procaryotic and Eucaryotic tRNA's with Leucine[a]

acylation system	pmol of [^{3}H]Leu acylated/min	
	-MAR	+MAR
rat liver	0.033	0.012 (65)
rat muscle	0.018	0.007 (59)
MOPC-41	0.060	0.022 (64)
yeast	0.109	0.085 (22)
wheat germ	0.208	0.154 (26)
S. antibioticus	0.069	0.070 (0)
E. coli	0.799	0.861 (0)
P. fluorescens	0.587	0.591 (0)

[a] Reaction mixtures contained 2 μM leucine (10 μCi of [^{3}H]-leucine per 100 μL of reaction mixture) and the following concentrations of tRNA and crude aminoacyl-tRNA synthetase, respectively, from the sources indicated: rat liver, 5.7 A_{260}/mL, 1.5 mg/mL; rat muscle, 5.1 A_{260}/mL, 1.2 mg/mL; MOPC-41, 4.9 A_{260}/mL, 1.3 mg/mL; yeast, 6.4 A_{260}/mL, 1.2 mg/mL; wheat germ, 6.9 A_{260}/mL, 1.2 mg/mL; *S. antibioticus*, 5.1 A_{260}/mL, 1.2 mg/mL; *E. coli*, 5 A_{260}/mL, 1.2 mg/mL; and *P. fluorescens*, 5.2 A_{260}/mL, 1.2 mg/mL. Incubation and processing were as described under Materials and Methods. Figures in parentheses represent the percent inhibition by marcaine (15.4 mM).

basic, acidic, aromatic, and sulfur containing) were examined. Crude rat liver aminoacyl-tRNA synthetase and total tRNA were used in these studies. Each of the amino acids tested was present at a concentration of 10 μM. It can be seen from the table that marcaine effectively inhibited the esterification of only some of the amino acids tested. Aminoacylation with leucine and isoleucine was strongly inhibited, while the drug had a lesser effect on acylation with valine and lysine. Marcaine was essentially without inhibitory effect on the acylation of all the other amino acids tested in the experiments of Table I.

Effects of Marcaine on the Acylation of Procaryotic and Eucaryotic tRNA's. Earlier results from this laboratory suggested that marcaine would inhibit aminoacylation in cell-free systems from mammalian tissues but not in a cell-free system from *E. coli* (Johnson & Jones, 1978). It was again of interest to confirm these results and to extend them to other eucaryotic and procaryotic organisms. Table II presents the results of experiments in which the effects of the drug on aminoacylation in cell-free systems from several procaryotic and eucaryotic sources were studied. It can be seen that marcaine does inhibit aminoacylation in yeast and wheat germ as well as in the mammalian tissues which were studied. In addition, the drug was without an inhibitory effect on aminoacylation in two bacterial systems (*P. fluorescens* and *S. antibioticus*) other than *E. coli*. These results provide support for the hypothesis that marcaine is a specific inhibitor of eucaryotic aminoacylation.

Table III: Partial Purification of Rat Liver Leucyl-tRNA Synthetase[a]

step	vol (mL)	protein (mg)	units[b]	sp act.[c]	purifn (x-fold)	% yield
crude extract	60	2520	101	0.04		100
200000*g* supernatant	57	1670	116	0.07	1.8	116
$(NH_4)_2SO_4$ ppt	9	666	64	0.10	2.5	63
Bio-Gel A.5m	27	200	58	0.29	7.3	57
DEAE-cellulose	30	25	40	1.60	40	40

[a] Starting with 12 g of rat liver. [b] One unit represents the formation of 1 nmol of [^{3}H]leucyl-tRNA. The leucine concentration for these assays was 100 μM. [c] Units/mg of protein.

Table IV: Kinetic Parameters for the Acylation of Rat Liver tRNA with Leucine or Isoleucine and Inhibition by Marcaine[a]

amino acid	substrate	K_m (mM)	V_{max} (pmol/min)	K_i (mM)
Leu	tRNA	0.00065	2.83	15.7
	ATP	3.57	1.43	11.9
	Leu	0.207	1.43	11.6
	Leu[b]	0.841[c]	1970[c]	9.3[c]
Ile	Ile[d]	0.036	0.72	18.6

[a] Reaction mixtures were prepared and incubated as described under Materials and Methods. Kinetic parameters were determined from the least-squares "best fit" equations derived from the data in Figures 2–4. [b] Kinetic parameters determined for the ATP-PP_i exchange reaction. [c] Average of values obtained at two different marcaine concentrations. [d] Kinetic parameters obtained using crude rat liver aminoacyl-tRNA synthetase.

Partial Purification of Rat Liver Leucyl-tRNA Synthetase. In order to learn more of the molecular effects of marcaine on aminoacylation, it was deemed necessary to perform experiments to examine the kinetics of marcaine inhibition. To avoid possible artifacts which might result from the use of crude aminoacyl-tRNA synthetase, we prepared a partially purified leucyl-tRNA synthetase for the kinetic studies. The results of the partial purification are summarized in Table III. It can be seen that the enzyme was purified about 40-fold relative to the crude extract with 40% recovery. The purification procedure actually removed 99% of the protein originally present in the crude rat liver extract. The partially purified enzyme was found to be free of significant quantities of contaminating protease and inorganic pyrophosphatase activities (data not shown).

Kinetics of Marcaine Inhibition of Aminoacylation. Kinetic analysis of marcaine inhibition of aminoacylation was performed as described under Materials and Methods. The results of these experiments are shown in Figures 2 and 3. It can be seen that the inhibition kinetics are noncompetitive when the substrates varied are tRNA and ATP (Figure 2). In contrast, competitive inhibition kinetics are observed when the leucine concentration is varied (Figure 3A). Because of the structural similarity between leucine and isoleucine, it was suspected that marcaine might also be a competitive inhibitor of aminoacylation with isoleucine. This suspicion was confirmed by the experiments depicted in Figure 3B in which marcaine inhibition of isoleucine acylation was studied. The kinetic parameters obtained from the data of Figures 2 and 3 are summarized in Table IV. The K_m values for each substrate are within the range reported for other amino-

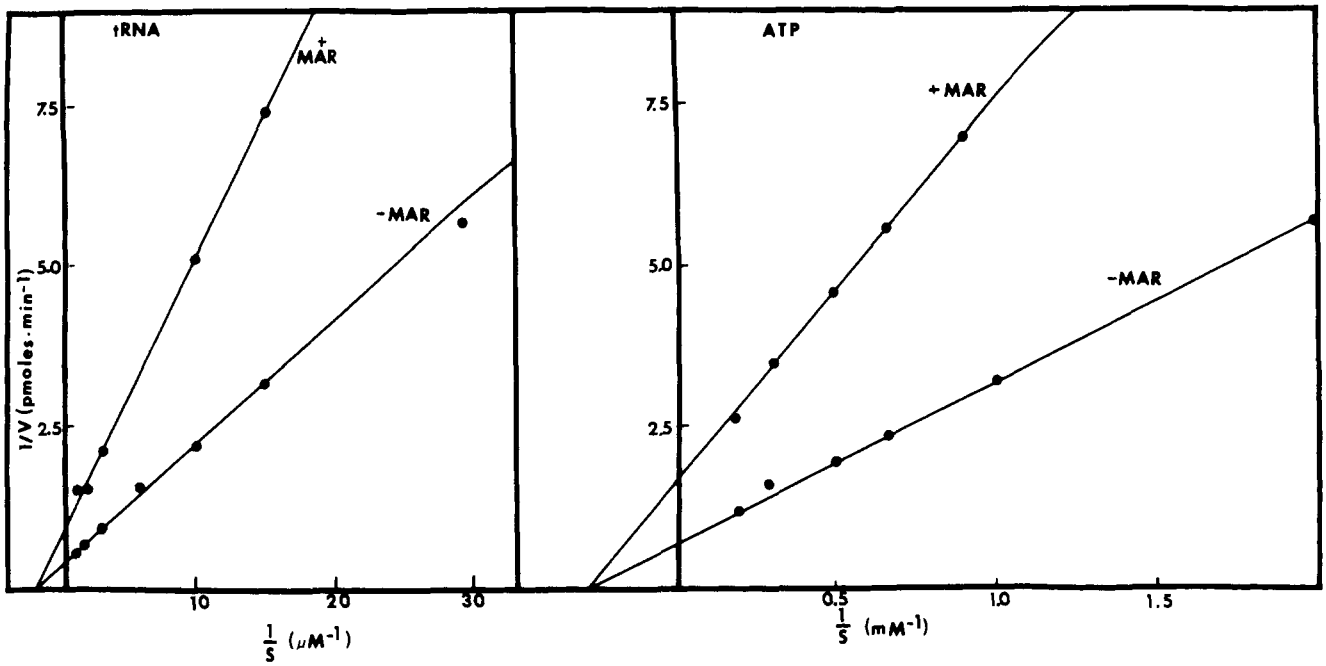

FIGURE 2: Kinetics of inhibition of aminoacylation of marcaine. Reaction mixtures were prepared, incubated, and processed as described under Materials and Methods. In the experiments depicted, concentrations of tRNA and ATP were varied. Data are plotted by the Lineweaver–Burk method. The marcaine concentration was 15.4 mM.

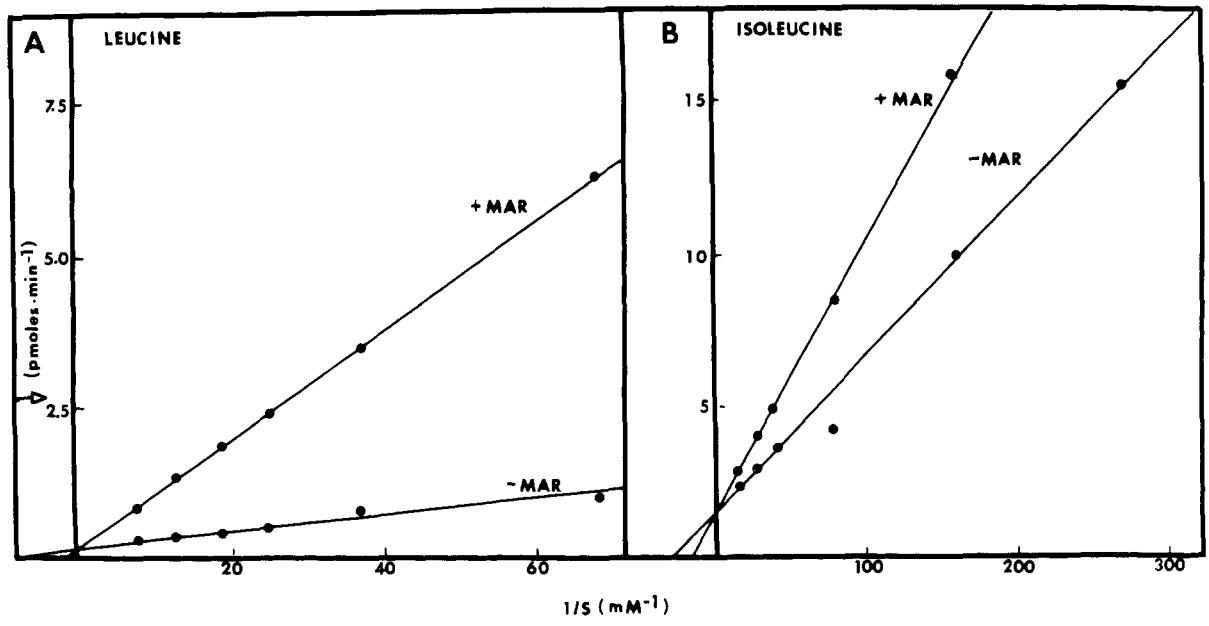

FIGURE 3: Kinetics of inhibition of aminoacylation by marcaine. Concentrations of leucine (A) or isoleucine (B) were varied in these experiments. In the studies with isoleucine a crude aminoacyl-tRNA synthetase preparation was used. Marcaine was present at 15.4 mM.

acyl-tRNA synthetases (Ofengand, 1977), and the V_{max} values for leucine aminoacylation differ by no more than a factor of 2 depending on the substrate varied. The variation in K_i for marcaine between 11.6 and 15.7 mM seems well within the range of experimental error.

Effects of Marcaine on Leucine-Dependent ATP–$^{32}PP_i$ Exchange. Given the foregoing results, it was of interest to determine whether marcaine inhibits leucine-dependent ATP–PP_i exchange as well as overall aminoacylation. The exchange reaction was carried out under conditions similar to those described by Calendar & Berg (1966) in the presence or absence of marcaine. Preliminary experiments showed that the drug did indeed inhibit the exchange reaction and a kinetic analysis of the effects of the drug is shown in Figure 4. It can be seen that marcaine is a competitor of leucine in the ATP–PP_i exchange reaction (presumably a measure of aminoacyl–adenylate formation) as well as in the overall aminoacylation reaction. Kinetic parameters determined by the exchange reaction are summarized in Table IV. The K_m for leucine was slightly larger than that determined in the overall aminoacylation reaction, while the V_{max} value for exchange was 1000-fold greater than the value for overall aminoacylation. A K_i value of 9.3 mM was determined for marcaine which is close to the values for K_i determined from aminoacylation studies (Table IV).

Effects of Marcaine Analogues on Leucine-Dependent ATP–$^{32}PP_i$ Exchange and Aminoacylation. The structure of marcaine is complex, and it was of interest to determine whether specific structural features of the drug were responsible for its observed inhibitory activity. The availability

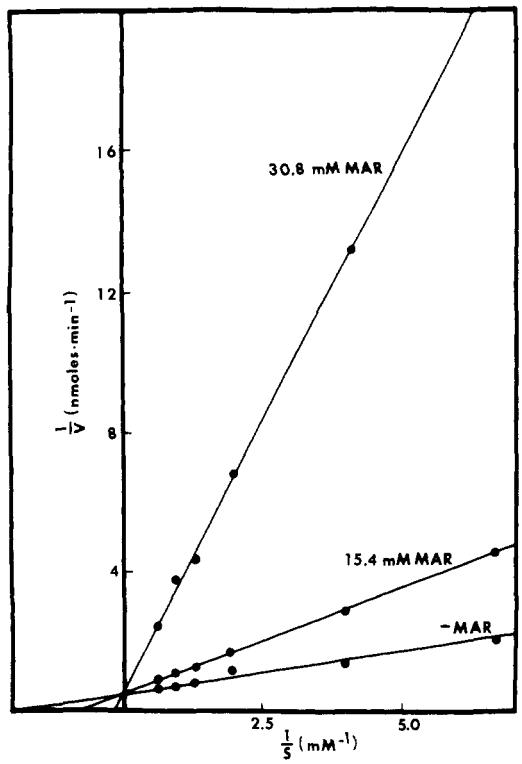

FIGURE 4: Kinetics of inhibition of leucine-dependent ATP–pyrophosphate exchange by marcaine. Reaction mixtures were prepared as described under Materials and Methods containing varying amounts of leucine. Marcaine concentration was 15.4 or 30.8 mM.

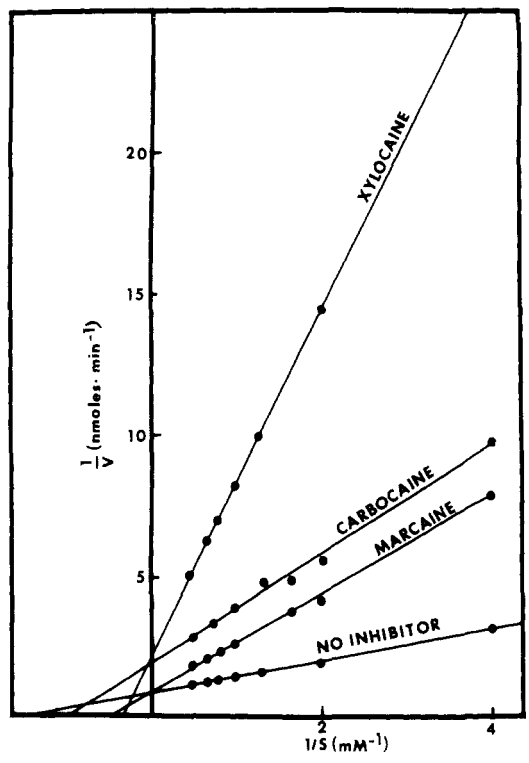

FIGURE 5: Kinetics of inhibition of leucine-dependent ATP–pyrophosphate exchange by marcaine, carbocaine, and xylocaine. Drug concentrations employed in these experiments were marcaine, 30.8 mM, carbocaine, 35.4 mM, and xylocaine, 36.9 mM.

of two structural analogues of marcaine, carbocaine and xylocaine (Figure 1), has made it possible to perform such an analysis. The effects of these two drugs on leucine-dependent ATP–$^{32}PP_i$ exchange are shown in Figure 5. It can be seen that both drugs inhibit the reaction but that the kinetics of inhibition are not the same as those exhibited when marcaine is used. Both carbocaine and xylocaine exhibit kinetics of the noncompetitive type in which both K_m and V_{max} are affected (Figure 5). Marcaine was competitive with leucine (Figures 3 and 4) and noncompetitive with tRNA and ATP (in the aminoacylation reactions, Figure 2). In the case of the latter two substrates, the presence of marcaine affected V_{max} but not K_m (Figure 2).

Because of the difference in inhibition kinetics exhibited by the three drugs, it was not possible to compare their inhibitory activity directly by determining K_i. Some indication of the relative inhibitory strength of the three drugs is shown by the data of Figure 6. In the experiments depicted, varying concentrations of each drug were added to reaction mixtures for aminoacylation and the percentage of inhibition was plotted against the molar concentration of drug added. On this basis, it is seen that marcaine was the most effective inhibitor of leucine acylation, xylocaine less effective, and carbocaine least effective (Figure 6).

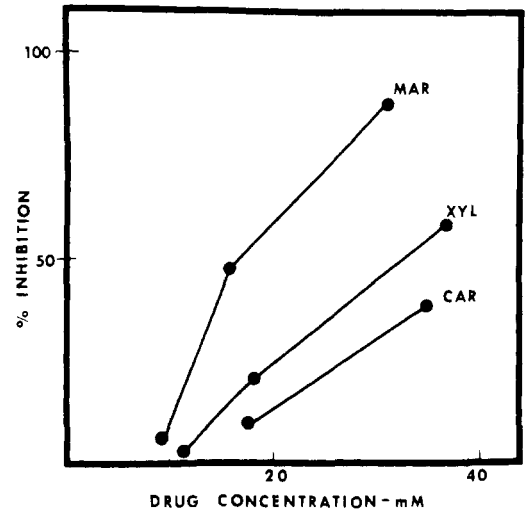

FIGURE 6: Effects of marcaine, carbocaine, and xylocaine on overall aminoacylation with leucine. Reaction mixtures were prepared as described under Materials and Methods containing 10 μM leucine. In the absence of inhibitor, 11 600 cpm of [^{3}H]leucine was esterified to 0.4 A_{260} unit of tRNA in 100 μL of reaction mixture. Partially purified leucyl-tRNA synthetase was used in these experiments.

Discussion

The data presented above demonstrate definitively that marcaine is a competitor of leucine both in overall aminoacylation and in the ATP–pyrophosphate exchange reaction. Although no marcaine-binding experiments were performed in the present study, the correspondence of the K_i values obtained when tRNA, ATP, and leucine concentrations were varied (Table IV) suggests that marcaine inhibits aminoacylation by binding to the aminoacyl site on the aminoacyl-tRNA synthetase. As predicted, marcaine also competes with isoleucine in the overall aminoacylation reaction (Figure 3). The data of Table I suggest that marcaine is less effective in inhibiting isoleucine acylation than leucine acylation, and this suggestion is supported by the kinetic data of Table IV. The K_i for inhibition of leucine acylation is 11.6 mM, while the corresponding value for isoleucine is 18.6 mM. It should be noted, however, that the studies with leucine were performed with a partially purified aminoacyl-tRNA synthetase preparation while in those with isoleucine a crude enzyme preparation was used. Nevertheless, the results obtained are consistent with the hypothesis that marcaine specifically inhibits leucine aminoacylation but also interacts with rat liver

isoleucyl-tRNA synthetase as a result of the structural similarity between the two amino acids.

It is important to note here that, in the inhibitor studies, ethanol was added to all reaction mixtures lacking marcaine (or other drug) to give final concentrations corresponding to those present in reaction mixtures containing varying amounts of the drug. Thus, the inhibitory effects which are observed are due to the marcaine (or other drug) itself and not to the ethanol in which it was dissolved.

The data of Table II demonstrate that marcaine is indeed a specific inhibitor of eucaryotic aminoacylation. The drug inhibits aminoacylation in cell-free systems derived from plants and yeast as well as in mammalian systems, but is completely without effect on aminoacylation in cell-free systems derived from both gram-positive and gram-negative bacteria. To the author's knowledge, this report represents the first discovery of a translational inhibitor specific for eucaryotic aminoacylation.

The use of marcaine analogues as potential inhibitors of aminoacylation has also provided some intriguing results. Although carbocaine and xylocaine both inhibit acylation of rat liver tRNA with leucine, the kinetics of inhibition are noncompetitive and of a type in which both K_m and V_{max} for the reaction are affected by the drugs. This type of inhibition (sometimes referred to as "mixed") was not observed with any substrate when marcaine was the inhibitor. Thus, it seems that the structural differences between marcaine and the other two drugs are sufficient to completely alter the pattern of inhibition. In the case of marcaine and carbocaine, the structural difference simply involves the removal of an *n*-propyl moiety from the side chain attached to the nitrogen atom of the pipecolic acid residue (Figure 1). This observation suggests that it is this *n*-propyl group which interacts with the aminoacyl-tRNA synthetase to produce the specific inhibition of the aminoacylation reaction observed with marcaine.

It should be emphasized finally that marcaine may have molecular effects other than those reported in this and the previous report from this laboratory (Johnson & Jones, 1978). The drug affects polypeptide chain elongation (Johnson & Jones, 1978), but other partial reactions of protein synthesis have not been examined. Although the drug has no apparent effect on RNA synthesis, it was observed in the previous study that 15.4 mM marcaine produced a greater degree of inhibition of protein synthesis in intact pieces of muscle than in a cell-free system derived from the same muscle (Johnson & Jones, 1978). This observation could be explained if the drug affects additional biochemical pathways whose activities are essential for continued protein synthesis in intact muscle. Studies on the effects of marcaine on mitochondrial function are in progress in this laboratory.

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