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Phenyl carbamates of amino acids as prodrug forms for protecting phenols against first-pass metabolism

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Summary

Various phenyl carbamate esters derived from amino acids, a dipeptide and amino acid esters and amides were prepared and assessed as potential prodrugs with the aim of protecting phenolic drugs against first-pass metabolism following peroral administration. The stability of the derivatives was studied in aqueous buffer solutions and in various biological media. The carbamates were rather stable in weakly acidic solutions but were hydrolyzed more facilely at physiological pH, the rates increasing greatly with decreasing pK_a value of the phenol. The hydrolysis of the amino acid carbamates was not catalyzed significantly by liver or intestinal wall enzymes but human plasma showed a marked catalytic effect. This latter effect could predominantly be ascribed to a catalysis exhibited by serum albumin. These results suggest that derivatization of phenolic drugs to form α-amino acid or dipeptide carbamate esters may be a potentially useful prodrug approach to reduce the extent of first-pass metabolism of the vulnerable phenol group.

Introduction

Several drugs containing a phenolic group show a limited and variable bioavailability following oral administration due to extensive first-pass metabolism in the gut and/or liver by glucuronidation, sulphation or methylation (George, 1981; Pond and Tozer, 1984). In some cases, bioreversible derivatization of the metabolically vulnerable phenol group to produce prodrug forms has proved to be a useful approach to reduce the extent of first-pass metabolism as demonstrated in mostly animal experiments. The examples described include anthranilate and salicylate esters of nalbuphine (Aungst et al., 1987), naltrexone (Hussain et al., 1987; Hussain and Shefter, 1988) and β-estradiol (Hussain et al., 1988), a pivaloyl ester of L-dopa (Ihara et al., 1989) and various carboxylate esters of the dopamine agonist N-0437 (Den Daas et al., 1990), various carbamate esters of the dopamine agonist (–)-3-PP (Thorsberg et al., 1987), fenoldopam (Brooks et al., 1990) and various 7-hydroxy-3-benzazepines (Hansen et al., 1991, 1992), and the bis-*N*,*N*-dimethylcarbamate ester of terbutaline (bambuterol) (Svensson and Tunek, 1988). As has been demonstrated with the anthranilate and salicylate esters of β-estradiol large species differ-

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ences exist, however (Lokind et al., 1991). A prerequisite for the usefulness of the prodrug approach is that demasking of the protective group occurs mainly in an organ other than the intestine or liver. If the prodrug-to-drug conversion occurs in the intestinal wall or liver during first-pass, the active parent phenol will subsequently be metabolized within the same organ. This is most often the case for enzymatically labile ester prodrugs and is the reason that the prodrug approach up to now has been met with only limited success.

A more promising and perhaps generally applicable approach to depress the first-pass metabolism of phenolic drugs may be development of prodrug derivatives where the conversion to the parent phenol occurs by non-enzymatic means, e.g. by chemical hydrolysis at the physiological pH of 7.4 or by an intramolecular reaction occurring with an appropriate rate at pH 7.4 and 37°C. To ensure passage of the prodrug in intact form through the stomach and upper intestine a suitable candidate should preferably be more stable at pH values lower than that of the blood.

Studies to exploit such a prodrug approach have been initiated in our laboratory and herein we report the stability of various phenyl carbamates (Table 1) in aqueous solution, plasma and liver homogenates. Most of the phenyl carbamates studied were derived from various amino acids, since it was thought that the ionized carboxylate moiety in these compounds might hinder an enzymatic hydrolysis. It is well known that carboxylic acid esters containing a negatively charged group near the ester bond are poor substrates for esterases (Nielsen and Bundgaard, 1987 and references cited therein). Such carbamate esters have not previously been synthesized except for the phenyl carbamate of L-phenylalanine (King et al., 1987) nor have they been studied in the prodrug context.

Materials and Methods

Apparatus

High-performance liquid chromatography (HPLC) was performed with a Merck-Hitachi apparatus consisting of an L-6000 pump, an L-4000 UV variable-wavelength UV detector, and a Rheodyne 7125 injection valve with a 20 μl loop. A deactivated Supelcosil LC-8-DB reversed-phase column (33 × 4.6 mm i.d.) (3 μm particles) from Supelco Inc., U.S.A., was used in conjunction with a Supelguard 200 precolumn. The pH measurements were made at the temperature of study using a Radiometer Type PHM 83 Autocal instrument. Elemental analysis was performed at Leo Pharmaceuticals, Ballerup, Denmark.

Chemicals

Chloroformates were purchased from Aldrich Chemie, Germany. The various amino acids and human serum albumin were obtained from Sigma Chemical Co., U.S.A. Buffer substances and solvents used were of reagent grade.

*Synthesis of the carbamate esters **I–XIII***

The phenyl carbamates of various amino acids (**I–IX** and **XI–XIII**) were prepared by reacting a phenyl chloroformate with the appropriate amino acid or its amide or ester derivative in ethyl acetate using the procedure described by Kruse and Holden (1985) for the preparation of benzyl carbamates of amino acids. In short, a mixture of the phenyl chloroformate (5 mmol) and amino acid (10 mmol) (finely powdered) in ethyl acetate (100 ml) was refluxed for 2 days with stirring. Upon cooling to room temperature the mixture was filtered and the filtrate evaporated in vacuo. The residue obtained was crystallized from ethanol-ether-petroleum ether. Physical and analytical data for the carbamates are given in Table 1.

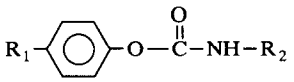
Phenyl *N*-ethylcarbamate (**X**) was prepared by adding ethyl isocyanate (1.19 ml, 10.6 mmol) to a mixture of phenol (0.94 g, 10 mmol) and triethylamine (1.40 ml, 10 mmol) in acetonitrile (20 ml). The mixture was stirred at room temperature for 6 h, filtered and the filtrate evaporated in vacuo. The residue obtained was crystallized from dichloromethane-petroleum ether, m.p. 48–49°C (reported m.p. 48–49°C (Kolbezen et al., 1954)).

Kinetic measurements

Hydrolysis in aqueous solutions The hydrolysis of the carbamate derivatives **I–XIII** was studied

TABLE 1

Physical and analytical data of various phenyl carbamates



Compound	R_1	R_2 [a]	M.p. (°C)	Formula [b]
I	H	CH_2COOH	98–100	$C_9H_9NO_4$
II	H	$CH(CH_3)COOH$	123–124	$C_{10}H_{11}NO_4$
III	H	$CH(CH_2CH(CH_3)_2)COOH$	oil	$C_{13}H_{17}NO_4$
IV	H	$CH(CH_2C_6H_5)COOH$	62– 63 [c]	$C_{16}H_{15}NO_4$
V	H	$CH(CH_2C_6H_5)COOH$ (D)	62– 63	$C_{16}H_{15}NO_4$
VI	H	$CH(CH_2C_6H_5)CONH_2$	175–176	$C_{16}H_{16}N_2O_3$
VII	H	$CH(CH_2C_6H_5)COOCH_3$	53– 54	$C_{17}H_{17}NO_4$
VIII	H	CH_2CH_2COOH	82– 83	$C_{10}H_{11}NO_4$
IX	H	$CH_2CONHCH_2COOH$	159–161	$C_{11}H_{12}N_2O_5$
X	H	C_2H_5	48– 49	$C_9H_{11}NO_2$
XI	OCH_3	$CH(CH_2C_6H_5)COOH$	100–101	$C_{17}H_{17}NO_5$
XII	Cl	$CH(CH_2C_6H_5)COOH$	118–119	$C_{16}H_{14}ClNO_4$
XIII	NO_2	$CH(CH_2C_6H_5)COOH$ [d]	–	–

[a] Unless otherwise specified the amino acid moiety was of the L-configuration.
[b] Elemental analyses (C, H and N) were within ±0.4% of the calculated values.
[c] Reported m.p. 62–63°C (King et al., 1987).
[d] The compound contained about 10% of *p*-nitrophenol.

in aqueous solutions at constant temperature (±0.2°C). The buffers used were hydrochloric acid, acetate, phosphate, borate and carbonate solutions. The buffer concentration generally used was 0.02 M. A constant ionic strength (μ) of 0.5 was maintained for each buffer solution by adding a calculated amount of potassium chloride.

The progress of hydrolysis of the carbamates was determined by using reversed-phase HPLC procedures capable of separating the compounds from their hydrolysis products. Mobile phase systems of 0.1% phosphoric acid containing acetonitrile (15–40% v/v) or methanol (10–15% v/v) were used, the concentration of acetonitrile and methanol being adjusted for each compound to give a retention time of 2–10 min. The effluent was monitored at 215 nm and the flow rate was 1.0 ml min^{-1}.

The reactions were initiated by adding 50–100 μl of a stock solution of the derivatives in acetonitrile to 10 ml of preheated buffer solution in screw-capped test tubes, the final concentration being 2×10^{-4}–5×10^{-3} M. The solutions were kept in a water bath at constant temperature and at appropriate intervals, samples were taken and chromatographed immediately. Pseudo-first-order rate constants for the degradation were determined from the slopes of linear plots of the logarithm of residual derivative against time.

Hydrolysis in biological media The degradation of the carbamates was studied at 37°C in 80% human plasma and in 10% rat liver homogenate. Compound **XII** was also studied in 10% rabbit gut and 10% pig liver homogenates. Compound **IV** was additionally studied in rabbit gut homogenate. The initial concentration of the compounds was about 10^{-4} M. The reaction mixtures were kept in a water bath at 37°C and at appropriate intervals samples of 250 μl were withdrawn and added to 500 μl of a 2% (w/v) solution of zinc sulphate in methanol-water (1 : 1 v/v) in order to deproteinize the samples and stop the reactions. After mixing and centrifugation for 3 min at 13000 rpm, 20 μl of the clear supernatant was analyzed by HPLC as described above.

Results and Discussion

Hydrolysis in buffer solutions

All phenyl carbamates studied underwent a quantitative degradation to the parent phenol in aqueous buffer solutions as revealed by HPLC analysis of the reaction solutions. At constant pH and temperature strict first-order kinetics was observed for the hydrolysis over several half-lives. At buffer concentrations less than 0.05 M no significant catalysis by the buffer substances used to maintain constant pH was observed.

At pH 7–10 and 37°C the rate of hydrolysis increased with increasing pH according to the following relationship:

$$k_{obs} = k_{OH} a_{OH} \quad (1)$$

where k_{obs} is the observed pseudo-first-order rate constant, a_{OH} denotes the hydroxide ion activity and k_{OH} is a second-order rate constant for the apparent specific base-catalyzed hydrolysis. The pH-rate profiles obtained for some carbamates are shown in Fig. 1 whereas the k_{OH} values are listed in Table 2 along with the half-lives of hydrolysis at pH 7.40.

In order to examine the influence of pH on the stability in acidic solution the hydrolysis of the carbamates **I, VIII** and **XII** was also studied at pH 2–7.4 at 60°C. The pH-rate profiles obtained are shown in Fig. 2. The shape of these profiles can

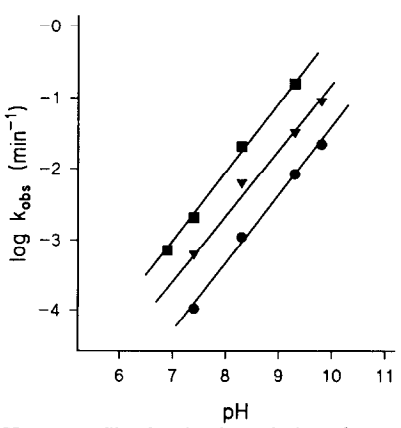


Fig. 1. pH-rate profiles for the degradation of compound **I** (●), **VI** (▼) and **XII** (■) in aqueous solutions at 37°C.

TABLE 2

Half-lives of hydrolysis of various phenyl carbamates and their k_{OH} values at 37°C

Compound	$t_{1/2}$ (h)			k_{OH} (M^{-1} min^{-1})
	pH 7.4 buffer	80% human plasma	10% rat liver homogenate	
I	116	20	> 50 [a]	1.7×10^{2}
II	183	20	> 50	1.1×10^{2}
III	196	5.7	> 50	1.0×10^{2}
IV	155	5.3	> 50	1.3×10^{2}
V	150	9.1	> 50	1.3×10^{2}
VI	6.4	0.7	0.04	3.1×10^{3}
VII	8.0	0.6	< 0.01	2.5×10^{3}
VIII	63	14	> 50	3.1×10^{2}
IX	8.0	2.9	10	2.5×10^{3}
X	61	1.8	26	3.2×10^{2}
XI	208	13	> 50	9.5×10^{1}
XII	19	0.5	19	1.0×10^{3}
XIII	0.03	–	–	6.0×10^{5}

[a] This notation implies that < 5% degradation was observed following incubation for 6 h.

be accounted for in terms of spontaneous or water-catalyzed reactions of undissociated and ionized carbamate and an apparent specific base-catalyzed reaction of the ionized form (Scheme 1 (compound **I**)). Mathematically,

$$k_{obs} = k_0 \frac{a_H}{a_H + K_a} + k_0' \frac{K_a}{a_H K_a} + k_{OH} \frac{K_a}{a_H + K_a} \quad (2)$$

where K_a is the ionization constant for the carboxylic acid group in the compounds. The values

TABLE 3

Rate data for the hydrolysis of various phenyl carbamates in aqueous solution at 60°C

Compound	k_0 (min^{-1})	k_0' (min^{-1})	k_{OH} (M^{-1} min^{-1})	pK_a [a]
I	1×10^{-5}	5×10^{-5}	1.5×10^{3}	4.4
VIII	5×10^{-6}	4×10^{-5}	2×10^{3}	4.5
XII	1×10^{-4}	4×10^{-4}	6×10^{3}	4.1

[a] Kinetically determined values.

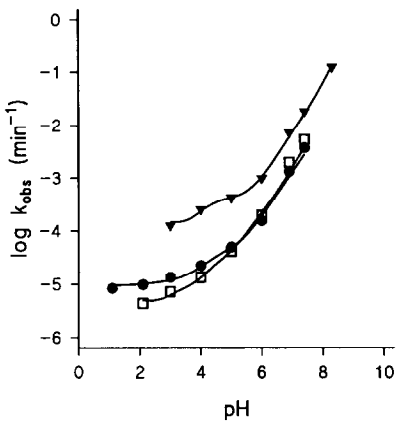
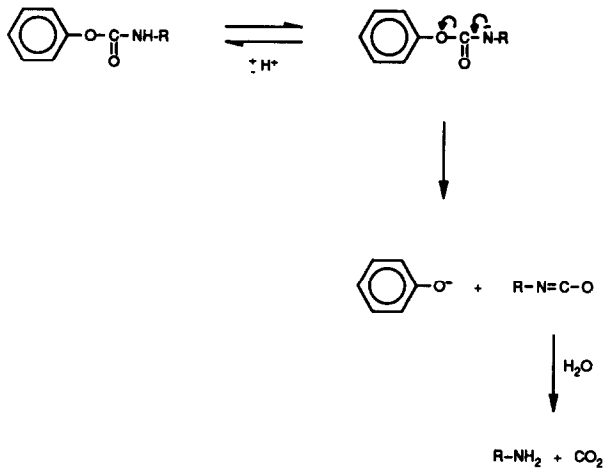


Fig. 2. pH-rate profiles for the degradation of compound **I** (●), **VIII** (□) and **XII** (▼) in aqueous solutions at 60°C.



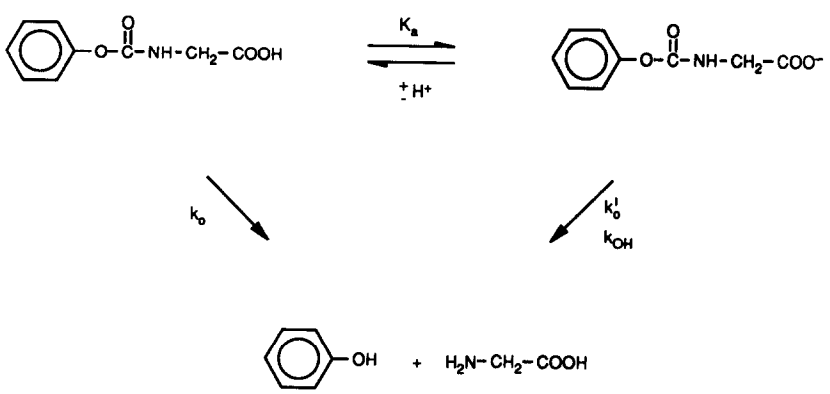
Scheme 2.

obtained for the various rate constants at 60°C are listed in Table 3.

The rate data show that the carbamates are rather stable in acidic aqueous solutions and that the ionized species are 4–8-fold more reactive than the species with an unionized carboxy group. This increased reactivity in the water-catalyzed hydrolysis may be due to intramolecular catalysis by the ionized carboxy group.

It has previously been established that the hydrolysis of monosubstituted carbamates of phenols in neutral and basic solution proceeds by an elimination-addition (E1cB) mechanism involving an intermediate formation of an unstable isocyanate (Scheme 2) (Vontor et al., 1972; Williams, 1972, 1973; Hegarty and Frost, 1973). Substituents in the phenolic leaving group have a considerable effect on the rate of hydrolysis (Williams, 1972). This is also the case for the phenyl carbamates derived from amino acids as seen from the Brønsted plot in Fig. 3 where log k_{OH} for the carbamates of L-phenylalanine (**I** and **XI–XIII**) has been plotted against the pK_a of the parent phenols. The slope of the straight line in Fig. 3 has a value of 1.30 which is identical to that (1.34) reported by Williams (1972) for substituted *N*-phenylcarbamates.

The reactivity of the phenyl carbamate esters of the various amino acids does not vary greatly with the structure of the amino acids. The reactivity increases, however, with increasing polar



Scheme 1.

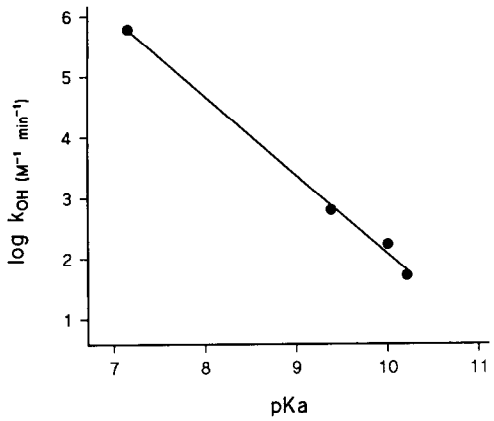


Fig. 3. Plot of log K_{OH} for the specific base-catalyzed hydrolysis of phenyl carbamate esters (**IV**, **XI–XIII**) against the pK_a value of the parent phenols.

effects within the *N*-substituent (Hegarty and Frost, 1973) and this effect may explain the increased reactivity of the amino acid amide and ester derivatives **VI** and **VII** as well as the dipeptide derivative **IX** relative to that of the amino acid derivative containing the strongly electropositive -COO^- group. All the latter compounds degraded at the carbamate bond to form phenol.

Hydrolysis in biological media

The susceptibility of the carbamate esters to undergo potential enzymatic hydrolysis was studied at 37°C in 80% human plasma and 10% rat liver homogenate (pH 7.4). The rates of hydrolysis of all carbamates in the plasma solutions were found to follow good first-order kinetics and the parent phenol was released in stoichiometric amounts. The observed half-lives of hydrolysis are listed in Table 2. As can be seen from the rate data, plasma catalyzes the rate of hydrolysis by factors ranging from 3 to 35. In the case of compound **VII** containing a methyl ester moiety HPLC analysis showed that the predominating reaction in plasma was cleavage of the carbamate ester bond to produce phenol and not cleavage of the ester bond to give compound **IV**.

In contrast to the catalytic effect exhibited by plasma, rat liver homogenate showed only a very small effect, if any, on the rate of hydrolysis of the amino acid carbamates. Less than 5% degradation was observed for these compounds upon incubation for 6 h (Table 2). The very rapid degradation of the amide **VI** and the ester **VII** in the liver homogenate was found to be due to cleavage of the terminal amide and ester groups, respectively, with formation of the free acid **IV**. This lack of significant liver enzyme-catalyzed hydrolysis of the amino acid carbamates is quite interesting and may imply that such derivatives may be able to pass through the liver without enzymatic attack and thus be able to protect the parent phenol against first-pass metabolism in the liver. This lack of liver catalysis is not confined to the rat liver since the half-lives of degradation of compound **XII** in both 10% pig liver homogenate (26 h) and 10% rabbit liver homogenate (16 h) were of the same order as that in buffer solution (19 h). It was similarly found that no enzymatic catalysis took place in a 10% rabbit gut homogenate. Following incubation for 6 h in this medium at 37°C compound **IV** showed less than 5% degradation.

The catalytic effect of human plasma was examined in more detail for compound **XII** and the *N*-ethyl carbamate **X**. Whereas the rate-accelerating effect of plasma increased greatly with increased plasma concentration for compound **X** (Fig. 4), the effect on the hydrolysis of the *N*-phenylalanine carbamate **XII** levelled off at plasma concentrations exceeding only about 3% (Fig. 5).

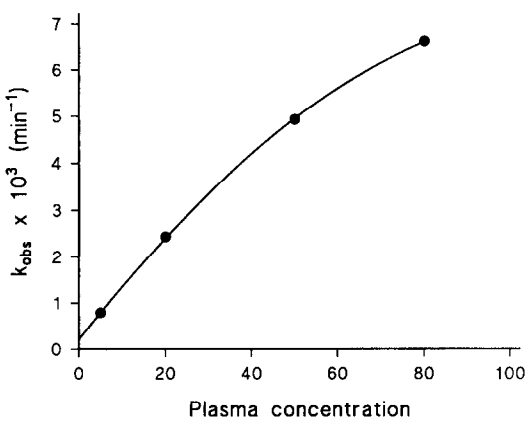


Fig. 4. Influence of human plasma concentration on the rate of hydrolysis of compound **X** (pH 7.4, 37°C).

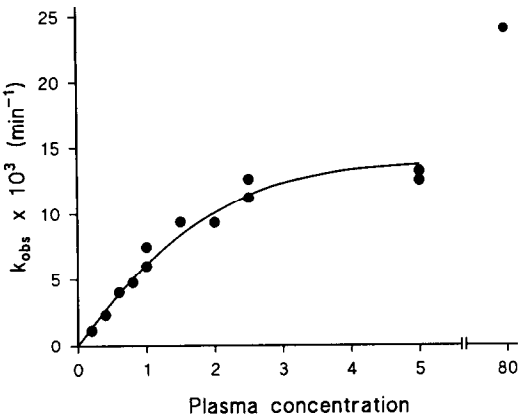


Fig. 5. Influence of human plasma concentration on the rate of hydrolysis of compound **XII** (pH 7.4, 37°C).

The predominant rate-accelerating component of human plasma for compound **X** was found to be albumin. As seen from Fig. 6 human serum albumin (HSA) exhibited a catalytic effect not much different from that of plasma. Since the concentration of HSA in plasma is about 4% a plasma concentration of 2.5% corresponds to an HSA concentration of 0.1%. Comparing the curves in Figs 5 and 6 shows that the levelling off of the curves in both cases takes place around these values and that the rates also correspond to each other. Thus, the value of k_{obs} is 0.011 min^{-1} in 2.5% plasma and 0.005 min^{-1} in a 0.1% HSA solution.

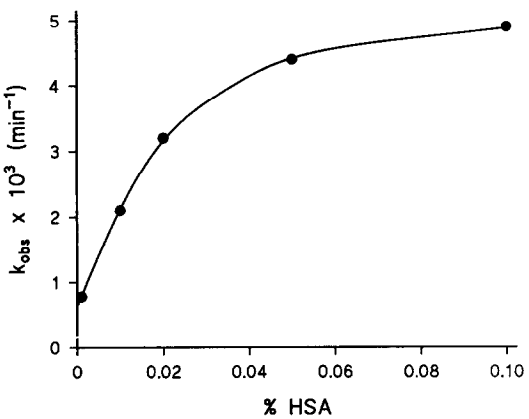


Fig. 6. Influence of human serum albumin (HSA) concentration on the rate of hydrolysis of compound **XII** (pH 7.4, 37°C).

HSA also showed a catalytic effect on the hydrolysis of the neutral *N*-ethyl carbamate **X** although to a lesser extent than for the amino acid carbamates. Thus, the half-life of hydrolysis of compound **X** was 6.2 h in a 4% HSA solution (pH 7.40) compared with 1.8 h in 80% human plasma and 61 h in a pH 7.4 buffer solution.

Further evidence for the predominant effect of serum albumin in the plasma catalysis was provided by the finding that the rate of hydrolysis of compound **XII** in human plasma containing physostigmine (10^{-4} M) was similar to that in plasma without this cholinesterase inhibitor. Pretreatment of the plasma solution at 65°C for 1 h before running the hydrolysis study also had only a slight influence on the hydrolysis rate. Thus, the half-life of hydrolysis of compound **XII** increased from 0.5 h in untreated plasma to 1.7 h in the heat-treated plasma whereas the half-life in pure buffer is 19 h.

Several years ago Casida and Augustinsson (1959) reported on a similar finding with 1-naphthyl *N*-methylcarbamate. The plasma-catalyzed hydrolysis of this phenolic carbamate was primarily ascribed to a catalytic effect of albumin whereas no influence was observed with several aromatic, aliphatic or choline plasma esterases. Several other investigators have also reported on the esterase-like activity of serum albumin, which is the most abundant protein in blood plasma, towards many different esters such as *p*-nitrophenyl esters (Kurono et al., 1979), aspirin derivatives (Kurono et al., 1982), oxazepam acetate (Aso et al., 1990) and gabexate (Ohta et al., 1986).

Conclusions

The results obtained suggest that derivatization of the phenolic group to form amino acid (or dipeptide) carbamate esters may be a potentially useful prodrug approach to reduce the extent of first-pass metabolism of phenolic drugs. The carbamates are rather stable in acidic and weakly acidic solutions but are hydrolyzed more facilely at physiological pH, the rates increasing greatly with decreasing pK_a value of the phenol. The

hydrolysis of the amino acid carbamates is not catalyzed significantly by liver and intestinal wall enzymes as well as by plasma enzymes but the hydrolysis is markedly catalyzed by human serum albumin. It should be noted, however, that simple phenols have been used as model compounds in this study. The possible utility of amino acid or dipeptide carbamate esters as prodrug forms can only be fully evaluated by studying such derivatives of each individual drug. Thus, it may be imagined that the catalytic effect of serum albumin on the rate of hydrolysis and hence bioconversion of the derivatives is greatly dependent on the structure of the drug moiety.

Acknowledgement

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