Potential effect of choline esters on choline-catalysed decarbamylation of dimethylcarbamoyl-acetylcholinesterase

Yun-Bae KIM, Chang-Hee JUNG, Seung-Ju CHOI, Won-Jun SEO, Seung-Hee CHA and Dai-Eun SOK*
Daejeon Machine Depot (no. 1-4-5), Daejeon P.O. Box 35, Daejeon 300–600, Korea

The choline esters potentiated the choline-catalysed decarbamylation of dimethylcarbamoyl-acetylcholinesterase in proportion to the length of acyl group, although esters containing an acyl chain longer than the hexanoyl group exhibited a corresponding decrease in the potentiation. In structural requirement analysis it was found that both the quaternary ammonium moiety and the ester bond were important for the effective acceleration of choline-catalysed decarbamylation. In general, the respective thiocholine ester was found to be more effective than the corresponding choline ester. Whereas the binding affinity (K_a) of choline in the decarbamylation was not significantly altered, the maximum decarbamylation rate ($k_{r(max)}$) of choline was greatly enhanced in the presence of choline esters or thiocholine esters. Along with the above observation, the isotope solvent effect, the effect of ionic strength and the antagonism studies demonstrate that the choline esters or thiocholine esters may interact with one of peripheral anionic sites, and thereby make the choline-catalysed decarbamylation more favourable.

INTRODUCTION

In earlier observations (Wilson & Alexander, 1962), it had been reported that the methylcarbamoyl-acetylcholinesterase, which was produced from the incubation of acetylcholinesterase with methylcarbamoylcholine, was decarbamoylated spontaneously. Later, the decarbamylation of dimethylcarbamoyl-acetylcholinesterase was reported to be accelerated by alcoholic compounds such as choline or 3,3-dimethylbutan-1-ol (Kitz *et al.*, 1970; Dawson, 1978). In particular, it was proposed that choline itself potentiated the decarbamylation by interacting with the central active site of the dimethylcarbamoyl-acetylcholinesterase. Subsequently, the choline-catalysed decarbamylation was observed to be affected by the allosteric effectors such as gallamine and tubocurarine (Rosenberry, 1975*a,b*; Pavlic & Wilson, 1978; Dawson *et al.*, 1981), which are known to interact with peripheral allosteric sites of the enzyme (Scheme 1*a*).

Also, decamethonium, a peripheral allosteric modifier, was observed to augment the re-activation of the phosphorylated acetylcholinesterase from erythrocytes by interacting with the regulatory site (Harris *et al.*, 1978). Thus the compounds that interact with peripheral binding sites of acetylcholinesterase were expected to modulate the catalytic function of the enzyme (Mooser & Sigman, 1974).

Earlier, it had been reported that at high concentrations acetylcholine decreased the spontaneous decarbamylation of the methylcarbamoylated acetylcholinesterase from electric eel by interfering with the anionic binding site (Wilson & Alexander, 1962), and it was proposed that the inhibition of decarbamylation by acetylcholine of high concentrations might be analogous to the substrate inhibition of acetylcholinesterase activity. Subsequently (Rosenberry, 1975*a,b*), the substrate inhibition of acetylcholinesterase (Scheme 1*b*) was observed to be caused by the interaction of the substrate with the allosteric regulatory sites, which were later supposed to be composed of multi-components involved in the regulation of enzyme activity (Zupancic, 1970; Harris *et al.*, 1978; Niday *et al.*, 1980). Thus acetylcholine in high concentrations was supposed to interact with one of the peripheral regulatory sites, and thereby inhibit the hydrolysis of the carbamoylated enzyme. However, it was

questioned whether even at low concentrations acetylcholine can exert an inhibitory effect on the decarbamylation. Instead, it was assumed that the spontaneous or choline-catalysed decarbamylation might be accelerated by low concentrations of acetylcholine, since acetylcholine in low concentrations had been assumed to accelerate its own hydrolysis (Zupancic, 1975). Moreover, the increase of non-polarity in the structure of choline esters was expected to affect the role of choline esters in the decarbamylation.

In this context, it seemed of interest to try to determine whether choline esters enhance the choline-catalysed decarbamylation and, if so, what types of binding site and binding properties were involved in the interaction. Furthermore, the understanding of the structural requirement for the effective acceleration of choline-catalysed decarbamylation would be extended to the design of effectors modulating the decarbamylation.

Our results demonstrate that the role of choline esters or thiocholine esters in the choline-catalysed decarbamylation is dependent on the size of the acyl group, and that the esters accelerate the choline-catalysed decarbamylation by interacting with one of the peripheral binding sites.

EXPERIMENTAL

Materials

Butyrylcholine chloride, propionylcholine iodide, laurylcholine iodide, palmitoylcholine iodide, acetylthiocholine iodide, butyrylthiocholine iodide, pyridostigmine bromide, gallamine triethiodide, 5,5'-dithiobis-(2-nitrobenzoic acid) and DEAE-Sephacel were obtained from Sigma Chemical Co. Choline chloride and acetylcholine chloride were procured from Merck, and 3,3-dimethylbutan-1-ol was from Aldrich Chemical Co. Pentanoylcholine iodide, hexanoylcholine iodide, heptanoylcholine iodide, octanoylcholine iodide, decanoylcholine iodide, isovalerylcholine iodide [m.p. 100 °C (decomp.)] and methacryloylcholine iodide (134.5–135.5 °C) were synthesized and identified as described by Kyi & Wilson (1957). Pentanoylthiocholine iodide, hexanoylthiocholine iodide, heptanoylthiocholine iodide and octanoylthiocholine iodide were prepared as described by Renshaw *et al.* (1938), and 2-(n-butoxy)ethyltrimethylammo-

* To whom correspondence should be addressed.

Fig. 1. Decarbamylation of dimethylcarbamoyl-acetylcholinesterase by choline, the respective choline ester or the combination with choline

Choline esters and/or choline were incubated with the carbamoyl-enzyme from mouse brain for 5 min in incubation buffer, pH 7.35, at 37 °C. A 25 μ l portion was used for the assay of enzyme activity as described in the Experimental section. (a) Butyrylcholine (BuCh); (b) pentanoylcholine (PeCh); (c) hexanoylcholine (HxCh); (d) heptanoylcholine (HpCh). ○—○, 0.3% Triton X-100 in incubation buffer; —, spontaneous decarbamylation.

Fig. 2. Effect of various choline esters on the choline-catalysed decarbamylation

The dimethylcarbamoyl-acetylcholinesterase was decarbamoylated by choline (1 mM) in the presence of the respective choline ester (1 mM) as described in Fig. 1 legend. Abscissa indicates the number of carbon atoms in normal acyl chain of choline esters.

Table 1. Comparison of the degree of decarbamylation of dimethylcarbamoyl-acetylcholinesterase by 3,3-dimethylbutan-1-ol, choline or the respective combination

Decarbamylation was performed and evaluated as described in the Experimental section.

Combination	Concn. (mM)	Decarbamylation (%)
Control	—	3.8 ± 1.66
3,3-Dimethylbutan-1-ol alone	1.0	5.8 ± 3.19
	10.0	4.2 ± 4.45
3,3-Dimethylbutan-1-ol (10.0 mM) + pentanoylthiocholine	1.0	6.8 ± 1.82
Choline alone	1.0	32.2 ± 2.18
+ Butyrylcholine	1.0	46.2 ± 1.60
+ Methacryloylcholine	1.0	20.8 ± 0.14
+ 2-(n-Butoxy)ethyltrimethylammonium	1.0	27.4 ± 0.15
+ Butyrylthiocholine	1.0	62.0 ± 3.14
+ Pentanoylcholine	1.0	49.9 ± 4.18
+ Isovalerylcholine	1.0	34.5 ± 0.87
+ 2-(n-Pentoxo)ethyltrimethylammonium	1.0	21.6 ± 0.43
+ Pentanoylthiocholine	1.0	64.9 ± 4.25
+ 2-(Dimethylamino)ethyl pentanoate	1.0	35.0 ± 2.74

Table 2. K_i values of various choline esters in the inhibition of soluble acetylcholinesterase from mouse brain

Acetylthiocholine-hydrolysing activity was measured in the presence of the respective choline ester, and the inhibition constant (K_i value) was obtained from Lineweaver-Burk-plot analysis (Zupancic, 1970). The respective choline ester was dissolved in 0.6% Triton X-100, and a 50 μ l portion was transferred into assay mixture (3 ml).

Compound	K_i (μ M)
Butyrylcholine	23.8 ± 1.44
Pentanoylcholine	72.3 ± 0.52
Hexanoylcholine	73.7 ± 1.39
Heptanoylcholine	32.4 ± 4.94
Octanoylcholine	16.6 ± 0.83
Decanoylcholine	5.2 ± 0.18
Laurylcholine	1.8 ± 0.02

nium iodide and 2-(n-pentoxo)ethyltrimethylammonium iodide and 2-(dimethylamino)ethyl pentanoate were prepared as described by Prager & Jacobson (1922).

Assay of acetylcholinesterase

Assay for acetylcholinesterase activity was performed at 25 °C by a slight modification of the method used by Ellman *et al.* (1961). The assay mixture (3.0 ml) contained 1.5 mM-acetylthiocholine, 1.0 mM-5,5'-dithiobis-(2-nitrobenzoic acid), 100 mM-NaCl and 100 mM-sodium phosphate buffer, pH 7.4. The change in absorbance at 412 nm was monitored continuously, and was confirmed to be linear for more than 30 min in the incubation with both membrane-bound and soluble acetylcholinesterase from mouse. Corrections were made for the non-specific hydrolysis of substrate, including non-enzymic hydrolysis. Under the experimental condition, the rate for the enzymic hydrolysis of butyrylthiocholine or other thiocholine esters with longer acyl chain was less than 2% of that for the hydrolysis of acetylthiocholine (Plummer *et al.*, 1984). Separately, Triton X-100 (0.05%) was added to the assay mixture to check the interference caused by the turbidity of membranes.

Preparation of dimethylcarbamoyl-acetylcholinesterase from mouse brain

Whole mouse brain was washed and homogenized in a Teflon/glass homogenizer in 15 vol. of 0.05 M-sodium phosphate buffer (incubation buffer), pH 7.35, containing 0.2 mM- CaCl_2 and 1.0 mM- MgCl_2 (Sakai *et al.*, 1985). The homogenate was centrifuged at 600 *g* for 10 min to remove fibrous tissues and blood contamination. Subsequently, the supernatant was centrifuged at 25000 *g* for 10 min at 4 °C, and the pellet was rehomogenized in the above incubation buffer to obtain 10% membrane-bound enzyme homogenate (Plummer *et al.*, 1975; Adamson, 1977).

This enzyme homogenate (approx. 0.6 activity unit/ml) was incubated with pyridostigmine bromide (final concentration 2 μ M) for 30 min at 37 °C under shaking, resulting in approx. 80–90% inhibition, and then washed at 4 °C twice with 15 vol. of saline to remove excess carbamate (Harris *et al.*, 1987). Finally, the inhibited enzyme pellet was rehomogenized in distilled water to make the 10% homogenate of the inhibited enzyme, and stored at 4 °C without significant change of carbamylation.

Separately, soluble acetylcholinesterase was obtained by treating the brain homogenate with 1% Triton X-100, and partially purifying the enzyme by using DEAE-Sephacel column (Rakonczay *et al.*, 1981).

Decarbamylation of the dimethylcarbamoyl-enzymes

Decarbamylation was started by addition of the inhibited enzyme homogenate (10 μ l) to the prewarmed (37 °C) incubation buffer (500 μ l) containing test compounds, and preserved at 37 °C under shaking. After 5 min decarbamylation, a 25 μ l portion was taken and subjected to the assay of acetylcholinesterase activity as described previously. The decarbamylation degree (%) was expressed as the percentage of the activity reactivated for 5 min out of the total amount of the initially inhibited enzyme, which was obtained by subtracting the enzyme activity at the start of decarbamylation from the activity after 2 h incubation of the inhibited enzyme with 2 mM-choline for full decarbamylation (Dawson, 1978). Separately, the first-order rate constant (k_1) was derived from the semi-logarithmic plots of percentage inhibition at three time intervals (5, 10 and 15 min). This experimental condition was designed to allow the concentration of thiocholine produced from 5.0 mM-acetylthiocholine during incubation to be lower than 10 μ M. Separately,

Fig. 3. Decarbamylation of dimethylcarbamoyl-acetylcholinesterase by the respective thiocholine ester or the combination with choline

Decarbamylation was carried out as described in Fig. 1 legend. (a) butyrylthiocholine (BuTCh); (b) pentanoylthiocholine (PeTCh); (c) hexanoylthiocholine (HxTCh); (d) heptanoylthiocholine (HpTCh).

Triton X-100 (0.3%) was included in the decarbamylation mixture to check the effect of turbidity. The result was expressed as the average of three or more experiments.

RESULTS

Since the choline esters such as acetylcholine, propionylcholine and butyrylcholine were found to enhance the choline-catalysed decarbamylation (Sok *et al.*, 1992), various synthetic choline esters containing different sizes of acyl group were evaluated for the potentiation of choline-catalysed decarbamylation. Fig. 1 shows that pentanoylcholine or hexanoylcholine significantly enhanced the choline-catalysed decarbamylation as observed with butyrylcholine, although the respective choline ester itself

showed only a small decarbamylation effect. In general, the potentiating effect of these choline esters reflected the additive or synergistic co-operation between choline and choline esters. On the other hand, the inclusion of heptanoylcholine or choline esters containing longer acyl groups resulted in the decrement of decarbamylation degree below that caused by choline alone. In a separate experiment where 0.3% Triton X-100 was added to the incubation mixture for decarbamylation, the same results were obtained, indicating that the inclusion of Triton X-100 in the reaction mixture used is not necessary (Fig. 1b).

In comparison (Fig. 2), the enhancing effect was observed to be remarkable with the choline esters containing acyl groups of intermediate size, but the long-acyl-group-containing choline esters such as palmitoylcholine, decanoylcholine and octanoyl-

Table 3. Degree of decarbamylation of dimethylcarbamoyl-acetylcholinesterase by the combination of choline (1.0 mM) and the respective thiocholine ester (1.0 mM)

Thiocholine esters with various lengths of acyl group were evaluated for the potentiation of choline-catalysed decarbamylation of dimethylcarbamoyl-acetylcholinesterase.

Combination	Concn. (mM)	Decarbamylation (%)
Control	—	3.5 ± 1.13
Choline alone	1.0	30.8 ± 1.96
+ Acetylthiocholine	1.0	28.4 ± 1.29
+ Butyrylthiocholine	1.0	59.5 ± 3.14
+ Pentanoylthiocholine	1.0	65.1 ± 3.01
+ Hexanoylthiocholine	1.0	63.2 ± 5.83
+ Heptanoylthiocholine	1.0	26.6 ± 1.83
+ Octanoylthiocholine	1.0	17.3 ± 0.85

Fig. 4. Variation of the first-order rate constant (k_r) for the decarbamylation of dimethylcarbamoyl-acetylcholinesterase

Choline (•), the combination of choline and 1.0 mM-choline ester (○, butyrylcholine; □, pentanoylcholine; △, hexanoylcholine) or the combination of choline and 1.0 mM-thiocholine ester (●, butyrylthiocholine; ■, pentanoylthiocholine; ▲, hexanoylthiocholine) was included in the incubation mixture. The rate constant was obtained as reported by Dawson (1978).

choline rather exerted an inhibitory effect on the choline-catalysed decarbamylation. Thus the enlargement of acyl chain length over six carbon atoms rather resulted in a decrease of the potentiating effect of choline esters.

Structure-activity relationship

Although 3,3-dimethylbutan-1-ol had been reported to catalyse the decarbamylation of the carbamoylated cholinesterase from bovine erythrocytes (Dawson, 1978), our results show that it is not comparable with choline in the decarbamylation of carbamoylated acetylcholinesterase from mouse brain (Table 1). Furthermore, the choline esters did not potentiate the 3,3-dimethylbutan-1-ol-catalysed decarbamylation. To investigate the structural requirement for the maximal potentiating effect of the choline esters in the choline-catalysed decarbamylation, various ester derivatives were synthesized and tested for the decarbamylation effect. As illustrated in Table 1, the quaternary

Fig. 5. Hanes plot for the decarbamylation of dimethylcarbamoyl-acetylcholinesterase in the presence of choline (○) or the combination of choline and 1.0 mM-pentanoylthiocholine (●)

[S] = concentration of choline; $v = (k_r - k_r')/k_r'$; k_r = observed first-order rate constant for decarbamylation in the presence of choline or the combination of choline and 1.0 mM-pentanoylthiocholine; k_r' = first-order rate constant for spontaneous decarbamylation = 0.0015 min⁻¹ (0.0013–0.0017 min⁻¹).

ammonium group was found to be important for the maximum potentiating effect (Roufogalis & Thomas, 1969), and the necessity of the ester bond is supported by the observation that the replacement of the ester group by an ether group led to the remarkable decrease of the potentiating effect. Additionally, it was observed that the n-butyryl group is more favourable than the branched or unsaturated acyl group. It is noteworthy to observe that the thioesters are more effective than the oxygen esters in potentiating the decarbamylation.

Although the reason why choline esters containing an acyl group longer than six carbon atoms are inhibitory on the choline-catalysed decarbamylation is not explained properly at this moment, it was found with the choline esters containing acyl chain longer than a hexanoyl group (Table 2) that the longer the acyl chain of the choline esters is, the greater the inhibitory role of the compound on the cholinesterase-catalysed hydrolysis of acetylthiocholine is. So, it is not a surprise to find out that pentanoylcholine and hexanoylcholine, possessing the higher K_i values in the inhibition of acetylcholinesterase activity, are more effective in potentiating the choline-catalysed decarbamylation (Fig. 2 and Table 2).

Co-operative interaction between choline and thiocholine esters

In the preceding data (Table 1), butyrylthiocholine was shown to be more effective than butyrylcholine in potentiating the choline-catalysed decarbamylation. Again, Fig. 3 shows that the thiocholine esters, butyrylthiocholine, pentanoylthiocholine or hexanoylthiocholine, greatly enhanced the choline-catalysed decarbamylation, although the thiocholine esters containing an acyl group of longer size (heptanoyl to octanoyl) lowered the decarbamylation degree of choline itself (Table 3). In a further study (Fig. 4) where the first-order rate constant (k_r) for the potentiated decarbamylation was plotted against the concentration of choline, it was observed that in general the thiocholine esters are more efficient than the choline esters, and that pentanoylthiocholine or hexanoylthiocholine enhanced the decarbamylation capability of choline 2-fold. Thus the combination of choline and pentanoylthiocholine made complete de-

Fig. 6. (a) Effect of pentanoylthiocholine on choline-catalysed decarbamylation of dimethylcarbamoyl-acetylcholinesterase, and (b) effect of choline on pentanoylthiocholine-catalysed decarbamylation of dimethylcarbamoyl-acetylcholinesterase

(a) Effect of pentanoylthiocholine (PeTCh) on choline-catalysed decarbamylation of dimethylcarbamoyl-acetylcholinesterase. The concentration of choline was varied, and that of PeTCh was fixed at 1.0 mM. (b) Effect of choline on pentanoylthiocholine (PeTCh)-catalysed decarbamylation of dimethylcarbamoyl-acetylcholinesterase. The concentration of PeTCh was varied, and that of choline was fixed at 1.0 mM. Under the conditions used, the enzymic or non-enzymic hydrolysis of PeTCh was negligible.

carbamoylation feasible within short time (approx. 10 min) under the condition used. On the basis of these observations, the co-operative interaction between choline and pentanoylthiocholine was more extensively studied. When the decarbamylation of the carbamoylated enzyme by choline alone or the combination of choline and pentanoylthiocholine was measured, and evaluated in terms of kinetic constants derived from a Hanes plot (Fig. 5), it was observed that the K_a value of choline was not greatly affected by pentanoylthiocholine whereas the $k_{r(max)}$ value was enhanced by 2-fold or more. Accordingly, it was assumed that pentanoylthiocholine exerted a directly enhancing effect on the choline-catalysed decarbamylation.

In a further experiment (Fig. 6), where the concentration of choline was varied and the concentration of pentanoylthiocholine was set at 1.0 mM, it was observed that the degree of decarbamylation by the combination was about 2-fold greater than that caused by choline alone over all the concentration range, in support of the above assumption concerning the potentiating role of pentanoylthiocholine in the choline-catalysed decarbamylation. Additionally, it was found that whereas choline exhibited the saturation kinetics at the concentrations higher than 10 mM, pentanoylthiocholine started to show the saturation pattern at concentrations lower than 0.5 mM. In an experiment (Fig. 7) where the effect of ionic strength was investigated, it was found that the binding constant (K_a) of choline in the decarbamylation was decreased 3-fold in low-ionic strength buffer, compared with that at high-ionic strength buffer, whereas pentanoylthiocholine enhanced the choline-catalysed decarbamylation to the same extent as observed in high-salt buffer. In a separate experiment (Table 4), where the decarbamylation by choline was investigated with $^2\text{H}_2\text{O}$ as a solvent, an isotope effect on the Michaelis constant was not observed, although the decarbamylation ratio ($v_{\text{H}_2\text{O}}/v_{^2\text{H}_2\text{O}}$) was found to be approx. 1.4. Meanwhile, the potentiating effect of

pentanoylthiocholine was not significantly altered in $^2\text{H}_2\text{O}$. In an additional study (Fig. 8), it was demonstrated that pentanoylthiocholine effectively decreased the decarbamylation effect of gallamine, a well-known allosteric peripheral effector, at higher concentrations, suggestive of competition between gallamine and pentanoylthiocholine towards the same binding site of the enzyme.

DISCUSSION

Choline was observed to enhance the decarbamylation of dimethylcarbamoyl-acetylcholinesterase as reported previously (Dawson & Poretski, 1985). However, 3,3-dimethylbutan-1-ol failed to enhance the decarbamylation, inconsistent with a former report (Dawson, 1978). This discrepancy may be due to differences in the enzyme source or the incubation conditions.

Although alcoholic and quaternary ammonium moieties are confirmed to be important for the structural requirement in the decarbamylation by choline as reported (Dawson, 1978), our observation suggests that the direct involvement of the nucleophilic alcohol in the decarbamylation may be less likely, since 3,3-dimethylbutan-1-ol, which had been reported to replace choline in nucleophilic decarbamylation, was found to be ineffective in our experimental conditions. Moreover, in buffer of low ionic strength the maximum decarbamylation effect of choline itself was not significantly altered, despite the observation that the nucleophilic activity decreased at lower ionic strength (Quinn, 1987). Further, thiocholine, a more potent nucleophile, was found to be one-third less effective than choline in decarbamylation.

In choline-catalysed decarbamylation, it was observed that among the short-acyl-group-containing esters the potentiating effect increased proportionally according to the length of acyl chain, with the maximal potentiation being observed with

Fig. 7. Effect of ionic strength on the decarbamylation of dimethylcarbamoyl-acetylcholinesterase by pentanoylthiocholine or combination of pentanoylthiocholine and choline (0.5 mM)

The experiment was performed as described in Fig. 6(b) legend, except that the ionic strength of the buffer was varied; (a) 2 mM-sodium phosphate buffer, pH 7.35; (b) the same buffer containing 150 mM-NaCl.

Table 4. Degree of decarbamylation of dimethylcarbamoyl-acetylcholinesterase by various concentrations of choline or the combinations of choline and pentanoylthiocholine in 2 mM-sodium phosphate buffer, pH 7.35, containing 150 mM-NaCl with distilled water (H_2O) or 2H_2O as solvent

In the respective case, the enhancement of decarbamylation by addition of pentanoylthiocholine of the respective concentration was similar between H_2O and 2H_2O buffer.

Combination	Concn. (mM)	H_2O	2H_2O
Control	—	3.4	0.0
Choline alone	20.0	55.0	39.6
	10.0	52.7	39.7
	5.0	50.4	37.8
	2.0	36.8	26.7
	1.0	34.4	25.3
	0.5	21.6	16.1
Choline (0.5 mM) + pentanoylthiocholine	0.5	41.9	29.3
	0.2	37.7	30.6
	0.1	30.3	21.9

pentanoylcholine or hexanoylcholine, although elongation of the carbon chain to longer than six carbon atoms resulted in a corresponding decrease of the potentiating effect as the acyl

Fig. 8. Effect of pentanoylthiocholine on gallamine-catalysed decarbamylation of dimethylcarbamoyl-acetylcholinesterase

The concentration of gallamine was varied, and that of pentanoylthiocholine was set at 100 μM in the low-ionic-strength buffer (2 mM-sodium phosphate buffer, pH 7.35).

chain length was increased. Thus the size of the acyl chain seems to govern the role of choline esters in the decarbamylation. Further, since the choline esters with branched or bulky acyl chains showing greater steric influence were less effective than their corresponding straight-chain isomers, it was inferred that the linear type of choline esters might be more favourable for the effective potentiation of choline-catalysed decarbamylation.

The studies concerned with the structural requirement indicate that both the quaternary ammonium moiety and the ester bond were essential for the effective potentiation of choline-catalysed decarbamylation, suggesting that the enzyme may have another selective binding site towards these choline esters, especially thiocholine esters. Previously it had been reported that the substrate inhibition of human erythrocyte acetylcholinesterase might be due to the interaction of acetylcholine or acetylthiocholine with one of multiple allosteric subsites of the enzyme (Niday *et al.*, 1980). At this moment, it is not clear whether the binding site for acetylcholine in the substrate inhibition corresponds to the binding site for these choline esters in decarbamylation. However, the observations that choline esters or thiocholine esters containing intermediate-size acyl groups effectively accelerate the choline-catalysed decarbamylation are suggestive of the selective interaction of the choline esters with one of the peripheral binding sites, distinct from the central binding site for choline. Moreover, the enhancing effect of gallamine, a peripheral accelerator, on the choline-catalysed decarbamylation was found to be antagonized by pentanoylthiocholine. Further support for the above suggestion comes from the observations that the maximum decarbamylation rate of choline is greatly enhanced by pentanoylthiocholine, whereas the K_a value of choline was not significantly changed in the presence of esters. And, although the binding affinity of choline was enhanced in lower-salt buffer as reported previously (Dawson, 1978), that of pentanoylthiocholine was not changed greatly. In addition, it was found that the rate of the choline-catalysed decarbamylation was affected by an isotope effect of solvent, but the potentiating effect of pentanoylthiocholine was not.

Although the subsites of the peripheral binding site were not investigated here, it seems that the peripheral binding subsite for the choline esters may be distinguished from that for the allosteric effectors such as gallamine or tubocurarine, on the basis of the observations that a decrease in ionic strength enhanced the

accelerated decarbamylation by the allosteric effectors (Dawson, 1978) but not that by choline esters, and that, whereas the ester linkage in the structure of choline esters is required for the effective accelerator of decarbamylation, the structure of the allosteric effectors contains the ether linkage.

Interestingly, the effects of choline esters on the choline-catalysed decarbamylation and the hydrolysis of acetylthiocholine by acetylcholinesterase differed according to the length of the acyl group; choline esters such as pentanoylcholine and hexanoylcholine, which efficiently accelerate the choline-catalysed decarbamylation, showed higher K_i values in the inhibition of acetylcholinesterase activity, whereas the choline esters with the acyl group of longer size (C_{12} – C_{16}), exhibiting a strong inhibition of the enzyme activity, rather decreased the choline-catalysed decarbamylation. These observations indicate that the interaction of the choline esters with the peripheral binding site may be characterized by a biphasic pattern of regulation, activatory or inhibitory, depending on the size of the acyl group, although it is not clear whether the interaction with the choline ester occurs on the same subsite of peripheral binding site or on the different subsites.

REFERENCES

- Adamson, E. D. (1977) *J. Neurochem.* **28**, 605–615
- Dawson, R. M. (1978) *J. Neurochem.* **30**, 865–870
- Dawson, R. M. & Poretski, M. (1985) *Biochem. Pharmacol.* **34**, 4337–4340
- Dawson, R. M., Poretski, M. & Upsher, C. M. (1981) *Neurochem. Int.* **3**, 405–409
- Ellman, G. L., Courtney, K. D., Andres, V. & Featherstone, R. M. (1961) *Biochem. Pharmacol.* **7**, 88–95
- Harris, L. W., Heyl, W. C., Sticher, D. L. & Broonfield, C. A. (1978) *Biochem. Pharmacol.* **27**, 757–761
- Harris, L. W., Talbot, B. G., Anderson, D. R., Lennox, W. J. & Green, M. D. (1987) *Life Sci.* **40**, 577–583
- Kitz, R. L., Braswell, L. N. & Ginsberg, S. (1970) *Mol. Pharmacol.* **6**, 180–221
- Kyi, Z.-Y. & Wilson, W. (1957) *Br. J. Pharmacol.* **12**, 38
- Mooser, G. & Sigman, D. S. (1974) *Biochemistry* **13**, 2299–2307
- Niday, E., Wang, C.-S. & Alaupovic, P. (1980) *Biochim. Biophys. Acta* **612**, 67–72
- Pavlic, M. R. & Wilson, I. B. (1978) *Biochim. Biophys. Acta* **523**, 101–108
- Plummer, D. T., Reavill, C. A. & McIntosh, C. H. S. (1975) *Croat. Chem. Acta* **47**, 211–223
- Plummer, D. T., Chai, M. S. Y. & Vidal, C. J. (1984) in *Cholinesterase* (Brzin, M., Barnard, E. A. & Sket, D., eds.), pp. 173–186, Walter de Gruyter, Berlin, New York
- Prager, B. & Jacobson, P. (1922) *Beilstein* **4**, 276–277
- Quinn, D. M. (1987) *Chem. Rev.* **87**, 955–979
- Rakonczay, Z., Mallol, J., Schenk, H., Vincendon, G. & Zanetta, J.-P. (1981) *Biochim. Biophys. Acta* **657**, 243–256
- Renshaw, R. R., Dreishbach, P. F., Ziff, M. & Green, D. (1938) *J. Am. Chem. Soc.* **60**, 1765–1770
- Rosenberry, T. L. (1975a) *Adv. Enzymol. Relat. Areas Mol. Biol.* **43**, 110–114
- Rosenberry, T. L. (1975b) *Adv. Enzymol. Relat. Areas Mol. Biol.* **43**, 190–196
- Roufogalis, B. D. & Thomas, J. (1969) *Mol. Pharmacol.* **5**, 286–293
- Sakai, M., Saisu, H., Koshigoe, N. & Abe, T. (1985) *Eur. J. Biochem.* **148**, 197–206
- Sok, D.-E., Kim, Y.-B., Cha, S.-H & Chung, Y.-S. (1992) *Neurochem. Int.* **20**, 201–205
- Wilson, I. B. & Alexander, J. (1962) *J. Biol. Chem.* **237**, 1323–1326
- Zupancic, A. O. (1970) *FEBS Lett.* **11**, 277–280
- Zupancic, A. O. (1975) in *Cholinergic Mechanisms* (Waser, P. G., ed.), pp. 321–325, Raven Press, New York

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