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Cholinesterase Inhibition in the Acute Toxicity of Alkyl-Substituted 2-Aminoethanols[1]

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The alkyl-substituted 2-aminoethanols have found widespread use in industry as absorbants, emulsifying agents, flotation agents, curing agents, and as chemical intermediates.

The acute toxicity of some of these compounds has been investigated by Smyth *et al.* (1954) and by Cornish (1965).

The 2-aminoethanols (Fig. 1A) may form quaternary amines (Fig. 1B) which are related to choline (Ch) (2-trimethylaminoethanol) (Fig. 1 C). Some of the substituted

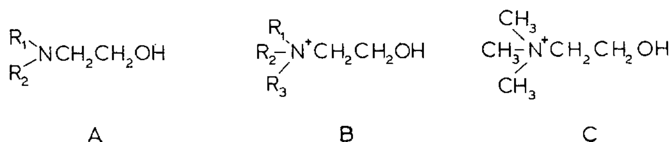

FIG. 1. Alkyl-substituted 2-aminoethanols.

2-aminoethanols can act as natural precursors in choline synthesis. du Vigneaud *et al.* (1946) showed that 2-methylaminoethanol (MeAE) and 2-dimethylaminoethanol (Me_2AE) could act as choline precursors, and Artom (1964) confirmed that phosphatidyl MeAE could be methylated by rat liver preparations. Bremer *et al.* (1960) have proposed that phosphatidylethanolamine is converted to choline by stepwise methylation.

Some compounds which are not normally precursors of choline may also enter into the metabolic pathways of choline synthesis. Bell *et al.* (1964) reported that 2-ethylaminoethanol (EtAE) and 2-diethylaminoethanol (Et_2AE) were methylated *in vivo* to their respective choline analogs.

Burgen *et al.* (1956) demonstrated that ethylcholine (EtCh), diethylcholine (Et_2Ch), triethylcholine (Et_3Ch), and *n*-butylcholine (BuCh) could be acetylated readily by a choline acetylase preparation from rat brain. The same enzyme preparation acetylated AE and MeAE to a slight extent. Dauterman and Mehrotra (1963) reported that a similar enzyme preparation could acetylate *N*-alkyl substituted choline analogs which contained two methyl groups. Mehrotra and Dauterman (1963) also reported that

[1] Portions of this paper were presented at the Society of Toxicology meetings in 1966 and 1967.

many *N*-alkyl analogs of acetylcholine could be hydrolyzed by rat brain cholinesterase.

It is apparent that many of the alkyl-substituted 2-aminoethanols are able to enter into some of the metabolic pathways of choline metabolism.

Many of the physiologic effects of the alkyl-substituted 2-aminoethanols also appear to relate to the normal functions of choline and its metabolites.

Hauschild (1943) reported reductions in blood pressure after EtAE and Et_2AE in cats. Kraatz *et al.* (1950) reported that Et_2AE stimulates the excised rabbit intestine or uterus and causes bronchoconstriction in isolated guinea pig lungs.

Me_2AE has been used clinically in the treatment of anxiety (Malitz *et al.*, 1967), on the rationale that it would increase endogenous acetylcholine.

Exposures of dogs to 12 ppm of AE vapor resulted in excitation followed by depression (Weeks *et al.*, 1960).

Intraperitoneal injection of Ch results in chromodacryorrhea, salivation, convulsions, and respiratory paralysis (Hodge, 1944).

Et_3Ch slows the release of acetylcholine and causes a muscular weakness similar to myasthenia gravis (Bowman and Hemsworth, 1965).

According to Bowman and Rand (1962), Ch and EtCh have primarily a depolarizing activity, Et_2Ch and Et_3Ch produce a presynaptic blockade, and BuCh and larger molecules have a curare-like action at the neuromuscular junctions.

Gebber and Volle (1965) have reported that Ch can stimulate the superior cervical ganglion of the cat directly, and Brestkin *et al.* (1965) reported that Ch could readily inhibit acetylcholinesterase. In addition, Krupka (1965) found that cholinesterase could be inhibited *in vitro* by various amines, including Me_2AE.

It becomes evident from the preceding discussion that the various alkyl-substituted 2-aminoethanols may produce a wide variety of neurologic effects and that some of these substances may produce several effects simultaneously.

The present study was undertaken as an attempt to correlate the acute oral and intraperitoneal toxicity of these compounds with their ability to inhibit cholinesterase activity *in vitro*. In selected instances *in vivo* cholinesterase inhibition was also investigated in brain and red cells of treated animals.

METHODS

The alkyl-substituted 2-aminoethanols were procured commercially from Eastman Organic Chemicals. Since they are normally quite alkaline, all were neutralized with HCl to a pH of 6.8–7.2 prior to use.

The choline analogs corresponding to some of these aminoethanols were also investigated. These choline analogs were prepared as the iodides by quaternization of the disubstituted 2-aminoethanols with the appropriate alkyl iodide. They were then purified by repeated recrystallization from alcoholic ether.

The acute oral and intraperitoneal toxicities of these compounds were studied in male Sprague-Dawley rats. The statistical analyses of the LD_{50}'s were done according to Weil (1952).

The *in vitro* studies utilized a purified enzyme preparation derived from bovine erythrocytes.[2] The enzyme activity was analyzed by a modification of the electrometric

[2] Sigma Chemical Co., St. Louis, Missouri

method of Michel (1949). This modification consisted of a titration of the buffer system with dilute acetic acid, so that the change in pH could be related to the amount of acetic acid liberated by the enzymatic hydrolysis of the acetylcholine substrate. During the analysis the pH varied from 7.7 to 7.3.

The brain and red cell cholinesterase levels were also determined in rats which were sacrificed 10–15 minutes after receiving an intraperitoneal dose of Me_2AE, EtAE, Bu_2AE, or Bu_2Ch. The whole rat brains were removed immediately after sacrifice and homogenized with an equal volume of physiological saline at 0°. Red cells were separated from the plasma by centrifugation and washed twice with physiological saline prior to analysis.

RESULTS

All the aminoethanols tested inhibited cholinesterase *in vitro* (Figs. 2–4). Aminoethanol and the monosubstituted 2-aminoethanols were the least inhibitory in character. Relatively high concentrations of inhibitor were required for significant inhibition (Fig. 2). The disubstituted 2-aminoethanols inhibited cholinesterase at a

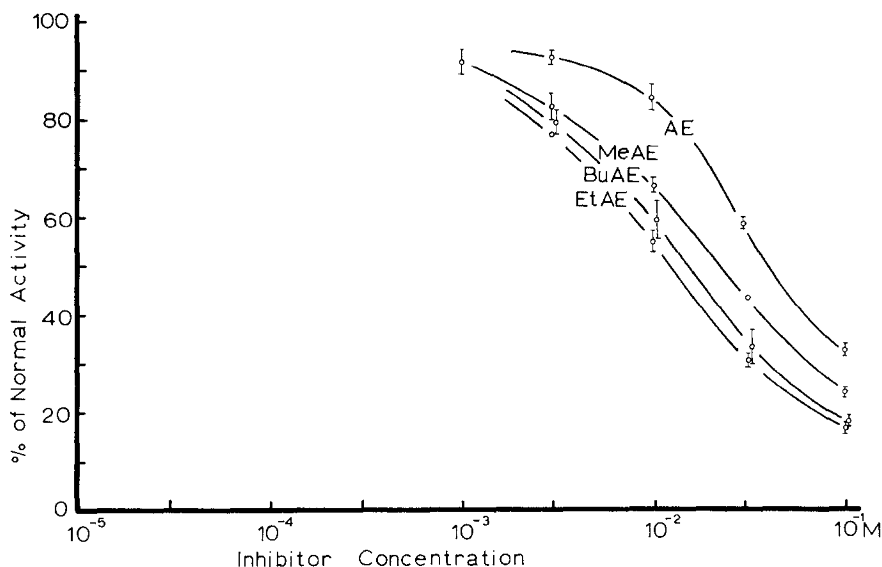

FIG. 2. Acetylcholinesterase inhibition by neutralized monoalkyl-2-aminoethanols.

concentration nearly an entire order of magnitude below that of the comparable monosubstituted compounds (Fig. 3). Diethanolamine (AE_2) occupied an intermediate position, and Bu_2AE was the most inhibitory of the disubstituted 2-aminoethanols.

Choline and the ethyl-substituted cholines inhibited at about the same level as some of the disubstituted 2-aminoethanols. The inhibition curves of Ch, EtCh, Et_2Ch, and Et_3Ch overlapped closely and have therefore been presented in general outline only (Fig. 4). The standard errors on these curves were comparable to those of the other cholinesterase inhibition curves in this study. BuCh and Bu_2Ch were the most inhibitory choline analogs tested.

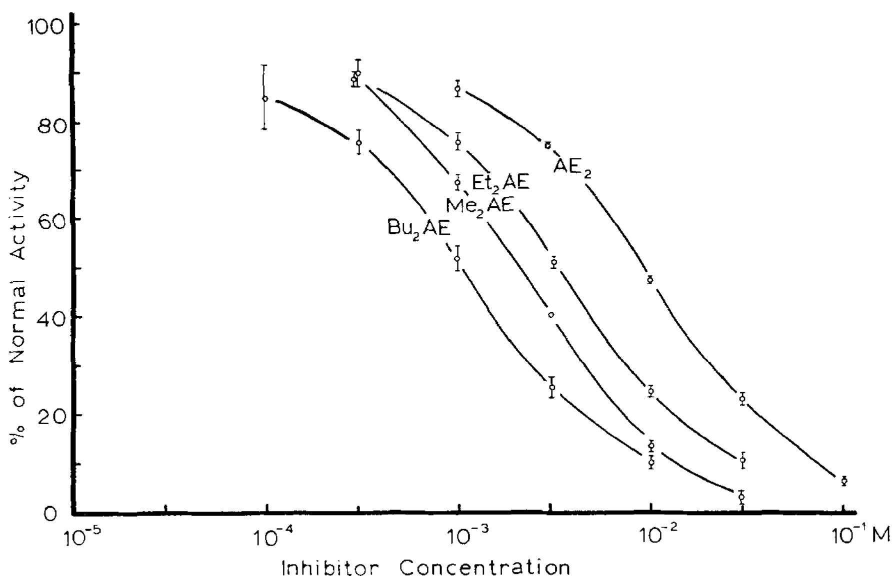

FIG. 3. Acetylcholinesterase inhibition by dialkyl-2-aminoethanols.

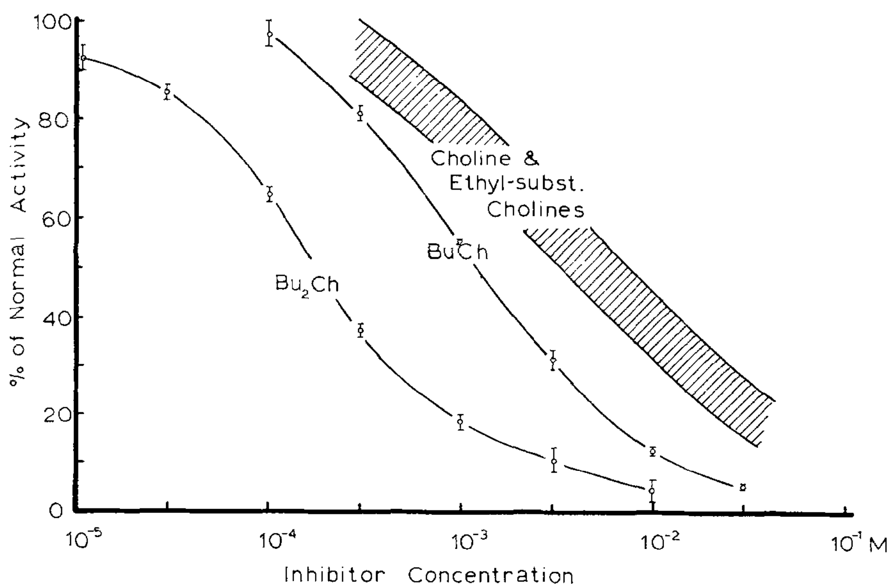

FIG. 4. Acetylcholinesterase inhibition by *N*-alkyl-substituted choline analogs.

In order to examine any quantitative relationship between *in vitro* cholinesterase inhibition and the acute toxicity of these compounds, their oral and intraperitoneal toxicities were determined (Table 1).

The oral LD_{50}'s are uniformly higher than the intraperitoneal LD_{50} values. Choline analogs exhibit a particularly large differential between oral and intraperitoneal

TABLE 1

LD_{50}'S OF SUBSTITUTED 2-AMINOETHANOLS

Compound	R_1	R_2	R_3	Oral LD_{50} (g/kg)	I.p. LD_{50} (g/kg)
AE	H	H	H	3.32 (2.71–4.07)[a]	0.981 (0.887–1.08)[a]
MeAE	CH_3	H	H	3.36 (2.37–4.75)	1.33 (1.06–1.69)
EtAE	C_2H_5	H	H	1.45 (1.19–1.77)	1.17 (1.02–1.34)
BuAE	C_4H_9	H	H	7.27 (6.60–8.00)	0.84 (0.65–1.21)
Me_2AE	CH_3	CH_3	H	6.00 (4.75–7.59)	1.08 (0.81–1.44)
Et_2AE	C_2H_5	C_2H_5	H	5.65 (3.49–9.14)	1.22 (0.88–1.71)
Bu_2AE	C_4H_9	C_4H_9	H	1.78 (1.33–2.39)	0.144 (0.091–0.219)
Ch	CH_3	CH_3	CH_3	6.64 (5.42–8.13)	0.400 (0.269–0.595)
EtCh	C_2H_5	CH_3	CH_3	6.93 (5.89–8.16)	0.297 (0.215–0.420)
Et_2Ch	C_2H_5	C_2H_5	CH_3	2.00 (1.37–2.99)	0.332 (0.271–0.407)
Et_3Ch	C_2H_5	C_2H_5	C_2H_5	1.00 (0.670–1.49)	0.181 (0.133–0.247)
Bu_2Ch	C_4H_9	C_4H_9	CH_3	7.88 (6.23–9.96)	0.089 (0.070–0.112)

[a] Figures in parentheses denote 95% confidence intervals.

toxicity, which is probably due to the slower absorption of these highly ionic quaternary amines from the gastrointestinal tract.

When the toxicity data and the inhibitory concentrations are compared on the basis of molar concentrations (Table 2), some interesting trends become evident. As the inhibitory capacity of the substituted 2-aminoethanol increases, there appears to be a

TABLE 2

CORRELATION OF *in Vitro* INHIBITION OF CHOLINESTERASE WITH THE ACUTE TOXICITIES OF SUBSTITUTED 2-AMINOETHANOLS

Compound	Inhibitor conc. producing 50% inhibition (mm/liter)	Oral LD_{50} (mm/kg)	I.p. LD_{50} (mm/kg)
AE	40	54.5[a]	15.9[b]
MeAE	23	44.7	17.7
EtAE	13	16.3	13.2
BuAE	15	61.9	7.17
Me_2AE	1.9	67.3	12.1
Et_2AE	3.1	48.2	10.4
Bu_2AE	1.1	10.3	0.831
Ch	6.2	28.8	1.73
EtCh	4.0	28.3	1.21
Et_2Ch	4.5	7.72	1.28
Et_3Ch	6.2	3.68	0.663
Bu_2Ch	0.18	25.0	0.282
BuCh	0.92		

[a] Correlation between median inhibitory concentration and oral LD_{50} $r = 0.378$, $p < 0.1$.
[b] Correlation between median inhibitory concentration and i.p. LD_{50} $r = 0.676$, $p < 0.01$.

concurrent increase in the intraperitoneal toxicity, resulting in a very significant correlation coefficient of $r = 0.676$. When the oral LD_{50} values are compared on the same basis, they generate a correlation coefficient of $r = 0.378$, which has only marginal significance.

When four of the aminoethanols were given intraperitoneally, they produced moderate but significant depressions in brain cholinesterase activities (Table 3). A significant depression in red cell cholinesterase activity was only noted for Me_2AE.

TABLE 3

CHOLINESTERASE LEVELS AFTER AN INTRAPERITONEAL LD_{50} DOSE OF SOME SUBSTITUTED 2-AMINOETHANOLS

Compound	Brain cholinesterase (percent of normal)	RBC cholinesterase (percent of normal)
Control	100 ± 2.4	100 ± 6.7
EtAE	83.5 ± 2.4^{a}	75.2 ± 7.5
Me_2AE	82.4 ± 5.8^{a}	54.9 ± 6.7^{a}
Bu_2AE	82.4 ± 5.8^{a}	100 ± 8.1
Bu_2Ch	88.0 ± 1.8^{b}	75.0 ± 10.1

[a] $p < 0.01$.
[b] $p < 0.05$.

DISCUSSION

The data presented in this paper indicate that neutralized *N*-alkyl substituted 2-aminoethanols inhibit cholinesterase *in vitro*. Inhibition increases as the degree of substitution and the molecular size of the nitrogenous head of the aminoethanol molecule increase. When the concentrations of substituted aminoethanols producing 50% inhibition *in vitro* are compared with their intraperitoneal LD_{50} doses on a molar basis, these two variables are found to correlate well on a statistical basis.

The increase in molecular size at the nitrogenous head has several important consequences. As the size of the substituent groups increases, the potential for van der Waals' interactions with the protein surface increases. Any increase in binding force with the enzyme near an active site would express itself in increased enzyme inhibition. When the log median inhibitory concentrations of the substituted 2-aminoethanols are compared on the basis of the number of substituent carbon atoms (Fig. 5), one notes that a great number of these points lie on or in very close proximity to a straight line.

In those cases, these data correlate closely with those of Bergmann (1955), who found linearly increasing inhibition of cholinesterase by compounds in the series $(CH_3)_3N(CH_2)_nCH_3$ as n was increased. In the series presented in Fig. 5, not all substituted 2-aminoethanols conform to the straight-line relationship. In the case of the ethyl-substituted quaternary amines, anomalous behavior in activity series had been noted previously by Collier and Exley (1963). The reasons for these anomalies are not entirely apparent. It is likely that forces other than van der Waals' interactions predominate disproportionately in these cases.

It has been suggested that coulombic attraction between the cationic head of a quaternary amine and an anionic site on the enzyme are of primary importance in the enzyme-substrate interactions (Wilson and Bergmann, 1950). As the nitrogenous head of the aminoethanol moiety in this study is substituted with an increasing number of

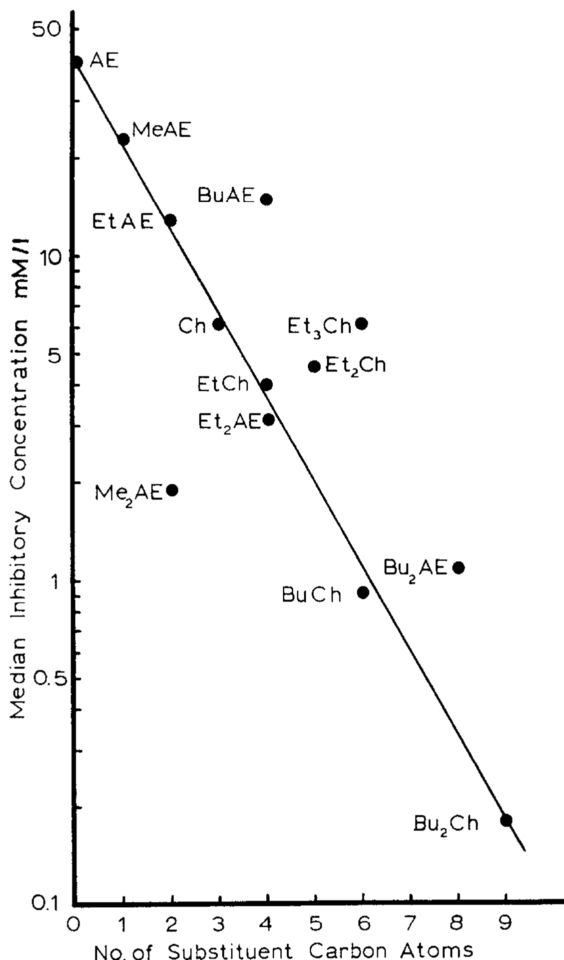

Fig. 5. Relationships of median inhibitory concentrations to the size of the substituent group on the nitrogenous head of the 2-aminoethanol molecule.

carbon atoms, there is an induction of electrons from the substituent chains toward the positive nitrogen atom. Therefore, the effective positive charge on the nitrogen atom decreases as the size and number of the alkyl substituents increases. As a consequence, the charge density of the cationic head decreases, and the coulombic attraction between this cationic head and the anionic site on the enzyme decreases also. In addition, steric effects of the substituent groups may further limit the approach of the two ionic sites, further limiting the degree of coulombic attraction.

It is, therefore, apparent that the inhibition of cholinesterase produced by the alkyl-substituted 2-aminoethanols cannot be readily explained by changes in anionic-cationic attraction. However, coulombic attractions combined with steric effects may well explain the divergences of some substituted 2-aminoethanols from the model based on van der Waals' interactions.

The three aminoethanols which were tested *in vivo* produced significant decreases in brain cholinesterase in rats within 15 minutes after an intraperitoneal LD_{50} dose. The observed decreases in brain cholinesterase were comparable in magnitude to those reported by Murphy (1967) in guinea pigs 15 minutes after 400 mg/kg i.p. of Malathion. While organophosphates often produce much higher levels of *in vivo* inhibition than those indicated here (Koppanyi and Karczmar 1951), the work of McIsaac and Koelle (1959) had indicated that the more lipid-soluble organophosphates readily inhibit both functional and reserve AChE, while the quaternary amines preferentially inhibit functional AChE. Since only the activity level of functional AChE has immediate physiologic significance, it is apparent that the measurement of total brain cholinesterase inhibition (functional + reserve) may underestimate the physiologic effect, especially in the case of quaternary amines.

The present data strongly suggest that cholinesterase inhibition is a factor in the acute toxicity of many alkyl-substituted 2-aminoethanols.

SUMMARY

The acute oral and intraperitoneal toxicity of a series of alkyl-substituted 2-aminoethanols was studied in relation to cholinesterase inhibition. All the 2-aminoethanols studied inhibited cholinesterase *in vitro*. The level of *in vitro* inhibition was related to the number of carbon atoms attached on the nitrogenous head of the 2-aminoethanol molecule. The median inhibitory concentrations correlate well with the LD_{50} data. Intraperitoneal LD_{50} doses of four alkyl-substituted 2-aminoethanols produced significant reductions in brain cholinesterase in rats.

ACKNOWLEDGMENTS

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