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Potential irreversible inhibitors of acetylcholinesterase: *N*-trimethyl-*N'*-iodoacetyl diaminoalkane iodides

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Potential irreversible inhibitors of acetylcholinesterase based on *N*-trimethyl-*N'*-iodacetyl diaminoalkane iodide $Me_3N^+[CH_2]_n$-$NHCO{\cdot}CH_2I{\cdot}I^-$, (I; n = 4) and (II; n = 2) have been prepared. (I) and (II) are both competitive inhibitors of the enzyme with K_i, $5{\cdot}9 \times 10^{-4}$M and $1{\cdot}2 \times 10^{-3}$M respectively. (I) is a partial agonist and (II) is a true agonist at the muscarinic receptor of guinea-pig ileum.

Many examples are known where a substrate or reversible competitive inhibitor of an enzyme when modified by the attachment of a suitable alkylating group becomes a specific irreversible inhibitor of the enzyme (Baker, 1964; Singer, 1967). The modified molecule is specifically bound at the active site by intermolecular forces and is correctly positioned for covalent bond formation with a functional group at (or near) the active site. We have designed compounds which are structurally related to acetylcholine based on *N*-trimethyl-*N'*-iodoacetyl diaminoalkane iodide, Me_3N^+-$[CH_2]_n{\cdot}NHCO{\cdot}CH_2I{\cdot}I^-$, (I; n = 4) and (II; n = 2) as potential irreversible inhibitors of acetylcholinesterase. The pharmacological action of (I) and (II) at the muscarinic receptor of guinea-pig ileum was also examined.

METHODS AND RESULTS

N-*Trimethyl*-N'-*iodoacetyl-1,4-diaminobutane iodide* (*I; n* = 4). Ethyl acetate (30·65 ml) was refluxed with 1,4-diaminobutane (85 g) for 48 h. The resulting mixture was distilled to give an oil (36·6 g), b.p. 119°/0·5 mm. Exposure of the oil in a thin film to air gave a white powder, m.p. 125·5–128°, which on sublimation gave white crystals, m.p. 132–133° of N-*acetyl-1,4-diaminobutane hemihydrate.* (Found; C, 52·0: H, 10·6; N, 19·9. $C_6H_{14}ON_2{\cdot}\frac{1}{2}H_2O$ requires C, 51·8; H, 10·9; N, 20·1%). ν_{max} (KBr), 3300 (NH), 1670 (C=O) cm^{-1}.

N-Acetyl-1,4-diaminobutane (5 g) in ethanol and iodomethane (9·5 ml) were added simultaneously to a stirred solution of sodium hydroxide (3·56 g) in ethanol at 0°. After the vigorous reaction had ceased, the mixture was maintained at 45–50° with stirring for 0·5 h and then kept overnight at 4°. The mother liquors were cropped and the organic material recrystallized from acetone to give white crystals (3·7 g), m.p. 179–179·5° of N-*trimethyl*-N'-*acetyl-1,4-diaminobutane iodide.* (Found: C, 36·3; H, 6·9; N, 9·5; I, 41·9. $C_9H_{21}ON_2I$ requires C, 36·0; H, 7·05; N, 9·3; I, 42·3%) ν_{max} (KBr), 3300 (NH), 1670 (C=O) cm^{-1}.

N-Trimethyl-*N'*-acetyl-1,4-diaminobutane iodide (1 g) was refluxed with alcoholic sodium hydroxide (25 ml, 1·4 g) for 5 h. The resultant oil was removed and refluxed with an excess of hydriodic acid for 0·25 h to give a reddish-brown solid which on

recrystallization from ethanol gave yellow crystals (0·52 g), m.p. 248–249° of N-*trimethyl-1,4-diaminobutane hydriodide iodide*, (Found; C, 21·60; H, 5·33; N, 7·40; I, 66·00. $C_7H_{20}N_2I_2$ requires C, 21·77; H, 5·22; N, 7·26; I, 65·73%). ν_{max} (KBr), 3450 (NH) cm^{-1}.

An alcoholic solution of sodium hydroxide (0·22 g) was added to a stirred solution (50 ml) of *N*-trimethyl-1,4-diaminobutane hydriodide iodide (1 g) in ethanol. Iodoacetyl chloride (1·06 g) was added to the cooled solution and the mixture stirred at 0° for 0·5 h. The mother liquors were cropped and the solid material obtained recrystallized from ethanol to give creamy-white crystals (0·48 g), m.p. 147–147·5° of N-*trimethyl*-N′-*iodoacetyl-1,4-diaminobutane iodide* (I). (Found: C, 25·4; H, 4·7; N, 6·3; I, 58·7. $C_9H_{20}ON_2I_2$ requires C, 25·4; H, 4·7; N, 6·6; I, 59·6%). ν_{max} (KBr), 3250 (NH), 1670 (C=O) cm^{-1}.

N-*Trimethyl*-N′-*iodoacetyl-1,2-diaminoethane iodide*. (*II*; $n = 2$). (II) was prepared from ethylenediamine by the general method described above and had m.p. 176–177°, (Found: C, 21·3; H, 4·1; N, 7·2; I, 63·9. $C_7H_{16}ON_2I_2$ requires C, 21·1; H, 4·05; N, 7·0; I, 63·8%).

(*I*) *and* (*II*) *as irreversible inhibitors of acetylcholinesterase*. The general inhibition procedure and the materials used have been described elsewhere (Beddoe & Smith, 1971).

A solution (19 ml) of each compound (1×10^{-2}M) was incubated with acetylcholinesterase (1 ml, 3·6 mg) in a medium containing sodium chloride (0·2M) at both pH 6·5 and pH 9·5. Samples (2 ml) of the mixture were periodically withdrawn and the remaining enzyme activity determined.

There was no decrease in the enzyme activity after incubation with (I) or (II) for 21 h at either pH.

(*I*) *and* (*II*) *as reversible inhibitors of acetylcholinesterase*. The rates of hydrolysis of acetylcholine over the concentration range $4–12 \times 10^{-4}$M by acetylcholinesterase (0·36 mg) were determined in a constant final reaction volume (20 ml) containing sodium chloride (0·2M) at pH 7·0 and 25°. The experiment was repeated in the presence of either (I) or (II) (2×10^{-3}M). The results were plotted according to Lineweaver & Burk (1934), i.e. 1/v *vs* 1/[S] where v is the rate of hydrolysis of the substrate (S).

The two linear curves obtained for each experiment in the presence and absence of the inhibitor intersected at the same point on the 1/v axis showing that each compound was a competitive inhibitor of the enzyme. The K_i values obtained for (I) and (II) were $5·9 \times 10^{-4}$ and $1·2 \times 10^{-3}$M respectively and the K_m value obtained under identical conditions but in the absence of the inhibitor was $1·65 \times 10^{-4}$M (*cf.* 2×10^{-4}M, Wright & Sabine, 1948).

Pharmacological action of (*I*) *and* (*II*) *on guinea-pig isolated ileum*. The tissue was employed as described previously using the Sanborn transformer and recorder (Beddoe, Nicholls & Smith: unpublished observations).

A cumulative dose-response curve for acetylcholine was initially obtained and then the response of the tissue to (I) and (II) was separately observed. The maximal heights of contracture of the tissue obtained with (I) and (II) were 60 and 100% respectively of the maximal contracture for the tissue with acetylcholine, suggesting that (I) is a partial agonist and (II) a true agonist at the muscarinic receptor of guinea-pig ileum. The mean value of the equipotent molar ratio for (II) by comparison with acetylcholine was 284.

DISCUSSION

(I) and (II) are reversible competitive inhibitors of acetylcholinesterase. The inhibition constants, K_i, were $5{\cdot}9 \times 10^{-4}$M and $1{\cdot}2 \times 10^{-3}$M respectively so that (I) is more firmly bound at the active site of the enzyme than (II).

This is to be expected since the higher homologue (I) possesses additional hydrophobic binding forces (Belleau, 1967). The absence of marked inhibition by compound (II), which structurally closely resembles acetylcholine, could be due to interaction with the anionic site and with a region away from the esteratic site. This view is supported by the similarity in the K_i values of (II) and tetramethylammonium ($1{\cdot}87 \times 10^{-3}$M, Kellett & Doggett, 1966; $2{\cdot}33 \times 10^{-3}$M, Belleau, 1965) which binds only to the anionic site.

Although both (I) and (II) are bound at the active site in an enzyme-inhibitor (EI) complex, neither alkylates a nucleophilic group on the enzyme in its vicinity with irreversible inhibition of the enzyme. The iodoacetyl group is a strong electrophile and is known to alkylate a variety of functional groups on proteins (Baker, 1964) so it would seem that in the EI-complex there is not a suitable or correctly positioned nucleophile on the enzyme in the vicinity of the iodoacetyl function for reaction.

O'Brien (1969) has recently indicated the presence of three binding sites for substrates and inhibitors of acetylcholinesterase designated α (anionic site), β and γ (hydrophobic sites), in addition to the esteratic site. In contrast to the results reported here, where binding by specific forces at the α-site is involved, we consider that the success of a wide range of chemical structures incorporating a phosphate or carbamate function as irreversible inhibitors at the esteratic site of the enzyme is due to binding at β- or γ-sites by non-specific van der Waal's and hydrophobic forces. The non-specificity of these binding forces would allow some degree of manoeuvrability in the location by these compounds of the required nucleophile (serine) on the enzyme surface (*cf.* Smith & Williams, 1967).

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