J. Pharm. Pharmac., 1976, **28**, 700–702 *Received March* 12, 1976

A new class of irreversible muscarinic antagonists: β-haloethylamine furoates

GERALD M. ROSEN* AND ELMER J. RAUCKMAN

Department of Physiology and Pharmacology, Duke University Medical Center, Durham, North Carolina 27710, *U.S.A.*

The synthesis of a new class of ultra-long acting muscarinic antagonists is described. Furthermore, it is noted that the stability of the, *in situ*, aziridinium ion is sufficiently great so that these agents can be used to study the effect of temperature upon the conformation of the muscarinic cholinoceptor. The inactivation kinetics of these receptors as well as the dissociation constants, K_d, for all probes are presented.

The value of irreversible blocking agents, such as dibenamine (Nickerson & Goodman, 1947) and benzilylcholine mustard (Gill & Rang, 1966) as tools to study the α-adrenoceptor and the muscarinic cholinoceptor remain questionable. However, these type compounds have been employed to investigate the effect of temperature on the molecular geometry about the receptor site, a phenomenon which can potentially regulate drug specificity (Ehrenpreis & Rosen, 1974).

As part of our study of the cholinoceptor, we have examined the effect of temperature on the binding of muscarinic antagonists to the guinea-pig ileum (Rauckman & Rosen, 1976). In this communication, we wish to report the synthesis and the mode of action of a series of irreversible muscarinic antagonists, 2-[(2-chloroethyl)methylamino]ethyl 5-substituted-2-furoate hydrochlorides.

METHODS

Rate of hydrolysis of aziridinium ion. The rate of hydrolysis of the aziridinium ion formed from IIa–c was determined by the thiosulphate titration method of Bartlett, Ross & Swain (1949).

2-[(2-hydroxyethyl)methylamino]ethyl 2-furoate (Ia). To a solution containing 14·5 g (121 mmol) *N*-methyldiethanolamine and 10 g potassium carbonate in 150 ml of dry benzene, was added, over 2 h, 7·9 g (60·5 mM) 2-furoic acid chloride. After the addition was completed, the reaction mixture was refluxed for 3 h, cooled and 100 ml of water was added. The layers were separated and the organic mixture was dried over anhydrous magnesium sulphate. Upon evaporation to dryness, the remaining oil was distilled giving 9·2 g (77%) of a colourless oil, B.P. 122–124° at 0·01 mm Hg.

* Correspondence.

In a similar manner, 2-[(2-hydroxyethyl)methylamino]ethyl 5-bromo-2-furoate (Ib) and 2-[(2-hydroxyethyl)methylamino]ethyl 5-methyl-2-furoate (Ic) were prepared.

2-[(2-chloroethyl)methylamino]ethyl 2-furoate hydrochloride IIa. To a solution containing 0·5 g (2·34 mmol) of the alcohol (Ia) in 30 ml of dry chloroform was added 4 ml of thionyl chloride. The reaction was warmed at 40° fo 2 h, cooled and evaporated to dryness. The remaining oil crystallized upon standing. The product was recrystallized from ethyl acetate–ethanol mixture giving 0·55 g (87%) of the desired product, m.p. 107–109°. Anal. Calcd for $C_{10}H_{15}NO_3Cl_2$: C, 44·8; H, 5·6; N, 5·2; Cl, 26·4. Found: C, 44·5; H, 5·65; N, 5·25; Cl, 26·3.

Similarly, 2-[(2-chloroethyl)methylamino]ethyl 5-bromo-2-furoate hydrochloride, m.p. 135–137°, Anal. Calcd for $C_{10}H_{14}NO_3Cl_2Br$: C, 34·6; H, 4·1; N, 4·0. Found: C, 34·6; H, 4·0; N, 4·1, (IIb) and 2-[(2-chloroethyl)methylamino]ethyl 5-methyl-2-furoate hydrochloride, m.p. 110–113°, Anal. Calcd for $C_{11}H_{17}NO_3Cl_2$: C, 46·8; H, 6·1; N, 5·0; Cl, 25·1. Found: C, 46·5; H, 6·2; N, 4·7; Cl, 24·6 (IIc) were prepared.

Studies on the muscarinic cholinoceptor

The guinea-pig ileum was prepared by conventional methods (Ambache, 1954; Paton & Rang, 1965). Contractions of the ileum were measured isotonically with a Harvard heart-smooth muscle transducer under a tension of 1 g. Contractions were elicited at equilibrium by injecting acetylcholine into the tissue bath every 2 min at 37°.

In a typical experiment, a dose-response curve was generated using acetylcholine at various concentrations. The [A50] of acetylcholine was used as the control dose for the remainder of the experi-

ment. At this point, a concentrated solution of the irreversible blocking agent, IIc, was added to the Tyrode bathing solution such that a final concentration of $2{\cdot}5 \times 10^{-7}$M of the inhibitor was obtained. Then, the [A50] of acetylcholine was injected, in the presence of the inhibitor, every 2 min for a 4 min exposure. At that point, the tissue was rapidly washed with bathing Tyrode solution not containing the inhibitor. We then repeated the dose-response curve and compared it with that of control.

In a second experiment, the above procedure was followed except that the length of exposure to the inhibitor was increased to 18 min. After washing the tissue as described above, the dose-response curve was repeated and was compared with the control curve. From these data, the rate of irreversible blockade at $2{\cdot}5 \times 10^{-7}$M as well as the rate of receptor regeneration was determined. In a similar manner, a family of pseudo first order rate constants, k_{app}, was obtained at various concentrations of the inhibitor (Fig. 1). Plotting the reciprocal of these rate constants, $(1/k_{app})$ as a function of the inhibitor concentration, (1/I) gave the dissociation constant, K_d (k_{-1}/k_1), for 2-[(2-chloroethyl)methylamino]ethyl 5-methyl-2-furoate, IIc (Kitz & Wilson, 1962). The dissociation constants for IIa–c are given with the formulae.

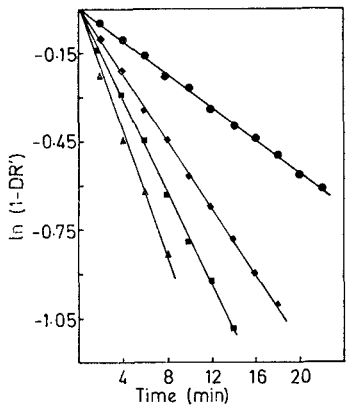
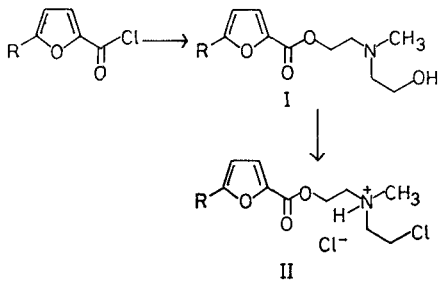


FIG. 1. A linear plot of the rate of irreversible blockade using 2-[(2-chloroethyl)methylamino]ethyl 5-methyl-2-fuorate, IIc, at ● 1×10^{-7}M, ◆ $2{\cdot}5 \times 10^{-7}$, ■ 5×10^{-7} and ▲ 1×10^{-6} is depicted. The rate constants, K_{app}, are 0·028, 0·060, 0·078 and 0·106 min^{-1}, respectively. Each point is an average of 4 independent experiments.

RESULTS AND DISCUSSION

Although the synthesis of a series of irreversible muscarinic blocking agents has been previously reported (Hiley, Young & Burgen, 1972; Young, Hiley & Burgen, 1972), their preparative sequence gave only minimal yields of the desired products. For this reason, we report the development, in high yields, of a new class of ultra-long acting muscarinic inhibitors. These compounds use an established pharmacologically active species as a carrier for a



		$K_d \times 10^{-7}$M
Ia, R = H	IIa, R = H	8·5 ± 0·32
Ib, R = Br	IIb, R = Br	4·6 ± 0·21
Ic, R = CH_3	IIc, R = CH_3	4·7 ± 0·18

potential alkylating moiety (2-haloethylamine). The preparation of these compounds is both straight forward and efficient. For example, reaction of 5-methyl-2-furoyl chloride with *N*-methyldiethanolamine followed by chlorination proceeds easily to give a 67% yield of the irreversible muscarinic antagonists, 2-[(2-chloroethyl)methylamino]ethyl 5-methyl-2-furoate hydrochloride (IIc).

We observed that in aqueous solution at pH 7·4, this compound like benzilylcholine mustard (Gill & Rang, 1966; Cuthbert & Young, 1973), rapidly forms the aziridinium ion, which is considered to be the alkylating species (Gill & Rang, 1966; Rosen, Ehrenpreis & others, 1971; Cuthbert & Young, 1973). Because of this observation, it is important to know the rate of hydrolysis of this ion. We have previously observed (Rosen, Ehrenpreis & Karoutsou, 1973) that a compound with an aziridinium ion which is hydrolysed too rapidly is of limited use since it cannot effectively alkylate the receptor sites. On the other hand, an aziridinium ion of high stability is not easily attacked by nucleophilic sites of the receptor macromolecule. Thus it is necessary to develop a probe whose aziridinium ion is of moderate stability in order to confer the proper degree of irreversibility. With this in mind, we determined that the $t_{\frac{1}{2}}$ at 30° for the hydrolysis of the aziridinium ion prepared from IIc was 50 min (Bartlett & others, 1949). In a similar manner, the $t_{\frac{1}{2}}$ for IIa and IIb was determined to be 35 and 45 min, respectively. Although the half-life of these aziridinium ions in IIa–c are

in vitro determinations, these data give a relative indication of their stability in the intact tissue.

With acetylcholine mustard (Robinson, Taylor & Young, 1974), the inactivation kinetics for the muscarinic receptors of the guinea-pig ileum by the irreversible antagonists, IIa–c, are consistent with the formation of a reversible drug receptor complex, D–R, followed at a much slower rate, by covalent bond formation.

$$D + R \underset{k_{-1}}{\overset{k_1}{\rightleftarrows}} D\text{–}R \xrightarrow{k_2} D\text{–}R' \xrightarrow{k_3} R' + D'' \quad (1)$$

This was shown to be so, since the contractions of the muscle can be restored to almost their original level (within 90% of the control height), if the tissue is washed soon after a blocking concentration (e.g. $2{\cdot}5 \times 10^{-7}$M) of the drug has been applied and the contractions of the muscle are diminished. On the other hand, if the tissue is exposed to the inhibitor at a concentration of $2{\cdot}5 \times 10^{-7}$M for 18 min and the drug rapidly removed from the system, the contractions of the muscle do not increase above the level observed at the point of wash out. This further suggests that the rate of irreversible bond formation, k_2, is much slower than k_1 and k_{-1}. With benzilylcholine mustard, Gill & Rang (1966) and Cuthbert & Young (1973) noted that $k_2 >>> k_{-1}$, then the free receptor fraction declines exponentially with time. For other muscarinic inhibitors (Gill & Rang, 1966; Cuthbert & Young, 1973; Robinson & others, 1974) as well as the furoate mustards, the rate of receptor regeneration, k_3, is very small. This conclusion is based on the observation that the blockade of the muscarinic contraction lasted longer than 6 h after removal o the drug from the tissue bath.

Since the concentration of the inhibitor is considerably greater than the number of receptor binding sites, we can rewrite equation 1 to read:

$$D + R \underset{k_{-1}}{\overset{k_1}{\rightleftarrows}} D\text{–}R \xrightarrow{k_2} D\text{–}R' \xrightarrow{k_3} D' + R'' \quad (2)$$

k_{app}

where k_{app} is the pseudo first order rate constant of irreversible receptor inactivation (Kitz & Wilson, 1962). The expression derived by Kitz & Wilson (1962) requires the determination of the fraction of receptors occupied by the irreversible inhibitor (DR'). This number can be obtained by using the following relation:

$$DR' = (dr - 1)/dr \quad .. \quad .. \quad (3)$$

where dr is the dose ratio by which the agonist concentration must be increased in order to produce a standard contraction of the muscle. Thus at various concentrations, one can obtain a family of rate constants, k_{app}. Plotting the reciprocal of these rate constants ($1/k_{app}$) as a function of the inhibitor concentration (1/I), one can obtain the dissociation constant, K_d ($= k_{-1}/k_1$), for the antagonist. For example, when 2-[(2-chloroethyl)-methylamino]ethyl 5-methyl-2-furoate, IIc, is employed as the antagonist, the dissociation constant, K_d, is $4{\cdot}72 \times 10^{-7}$M. The dissociation constants for the irreversible muscarinic antagonists, IIa–c are listed with the formulae.

REFERENCES

AMBACHE, N. (1954). *J. Physiol. Lond.*, **125**, 53P.

BARTLETT, P. D., ROSS, S. D. & SWAIN, C. G. (1949). *J. Am. chem. Soc.*, **71**, 1415–1419.

CUTHBERT, A. W. & YOUNG, J. M. (1973). *Br. J. Pharmac.*, **49**, 498–505.

EHRENPREIS, S. & ROSEN, G. M. (1974). *Nature*, **250**, 576–578.

GILL, E. W. & RANG, H. P. (1966). *Mol. Pharmac.*, **2**, 284–297.

HILEY, C. R., YOUNG, J. M. & BURGEN, A. S. V. (1972). *Biochem. J.*, **127**, 86P.

KITZ, R. & WILSON, I. B. (1962). *J. biol. Chem.*, **237**, 3245–3249.

NICKERSON, M. & GOODMAN, L. S. (1947). *J. Pharmac. exp. Ther.*, **89**, 167–185.

PATON, W. D. M. & RANG, H. P. (1965). *Proc. R. Soc. B.*, **163**, 1–44.

RAUCKMAN, E. J. & ROSEN, G. M. (1976). *Biochem. Pharmac.*, **25**, 1324–1332.

ROBINSON, D. A., TAYLOR, J. G. & YOUNG, J. M. (1974). *Br. J. Pharmac.*, **50**, 463P.

ROSEN, G. M., EHRENPREIS, S. & KAROUTSOU, A. (1973). *Archs int. Pharmacodyn. Thér.*, **204**, 242–251.

ROSEN, G. M., EHRENPREIS, S., MITTAG, T. W. & STUBBINS, J. F. (1971). *J. medl Chem.*, **14**, 514–516.

YOUNG, J. M., HILEY, C. R. & BURGEN, A. S. V. (1972). *J. Pharm. Pharmac.*, **24**, 950–954.