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Acetylcarbocholine and acetylsilicocholine: directly or indirectly acting cholinergic spasmogens?

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The cholinergic and anticholinergic actions of nitrogen-free isosteres of acetylcholine and benzilylcholine are described. Esters of two kinds of choline analogues, carbocholine and silicocholine, were used. The spasmogenic activity of acetylcarbocholine and acetylsilicocholine on the guinea-pig ileum was identified as an indirect cholinergic action, in contrast to the direct cholinergic action of furtrethonium and the mainly noncholinergic action of barium ions. In addition to this indirect cholinergic action, both esters show a weak anticholinergic and a weak noncompetitive "papaverine-like" spasmolytic activity. The corresponding benzilyl esters, although without an onium group, are relatively potent anticholinergic compounds.

GRADUAL elimination of the ester group-bearing side-chain of the acetylcholine molecule results in a gradual increase in the dose required to induce a response, such as contraction of the isolated gut of the rat. However, a maximum response equal to that of acetylcholine can still be obtained as long as the quaternary ammonium group, tetramethyl ammonium, is maintained. Elimination of the onium group, giving for example ethyl acetate, results in a loss of the cholinergic action. Ethylation of the onium group in cholinergic compounds as a rule results in a decrease or loss of the cholinergic intrinsic activity. These observations emphasize the significance of the trimethylammonium group in acetylcholine for its cholinergic action (Ariëns, 1964, 1965, 1966a, b; Ariëns & Simonis, 1960, 1964).

For anticholinergic compounds, where only an affinity to and no intrinsic activity on the cholinergic receptors is required, the quaternary ammonium group is less critical and ethylation or a change to a tertiary amino-group does not abolish the anticholinergic action. Even elimination of the onium group does not abolish anticholinergic action, as was demonstrated in the investigation of various nitrogen-free analogues of anticholinergic drugs of the benzilylcholine type (see Table 1 in which the anticholinergic activity is expressed as pA_2 values). The *N*-free anticholinergic compounds, although less active than their choline analogues, can nevertheless still be regarded as potent anticholinergics (Funcke, Rekker & others, 1959, 1960; Ellenbroek, 1964; Ariëns, 1965, 1966a, b, c).

The blocking activity of the benzilylcholine type of anticholinergic compounds is based mainly on the interaction of the ring-bearing acyl moiety to the accessory receptor areas. These are areas with which large acyl moieties will bind and they are located in the immediate vicinity of, but are not a part of, the cholinergic receptors in a strict sense. There is good evidence for identifying the mechanism of action of the *N*-free anticholinergic compounds and their analogues as an interaction with the same accessory receptor areas (Ariëns, 1965, 1966a, b, c). The elimination

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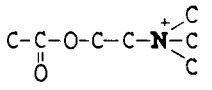
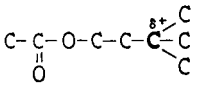
of the rings from the acyl moiety results in a strong decrease in the affinity (the pA_2 values) of these anticholinergic compounds for their receptors (see Table 1). As reported earlier (Ariëns, 1965, 1966a, b, c; Burgen, 1965) an unexpected finding was that 3,3-dimethylbutyl acetate, an isostere of acetylcholine called acetylcarbocholine, was spasmogenic on the isolated gut of the rat or guinea-pig, which might indicate a cholinergic action for this compound. In addition the same is found to hold true for acetylsilicocholine (trimethylsilylethylacetate*). The maximal contraction obtained with these compounds in cumulative concentration-response curves is smaller than that obtained with acetylcholine itself and cholinergic

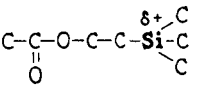


Cl^-

acetylcholine



acetyl"carbocholine"



acetyl"silicocholine"

compounds such as furtrethonium. If tested on the isolated gut of the guinea-pig the maximal contractions for acetylcarbocholine are 40–100% and the maximal contractions for acetylsilicocholine are 20–80% of those obtained with furtrethonium. If tested on the isolated gut of the rat these figures are 50–100% and 50–100% respectively. This may imply that acetylcarbocholine and acetylsilicocholine have to be classified as partial agonists (Table 1). Acetylcarbocholine was studied extensively by Whittaker (1954) for its action on acetylcholinesterase. In a series of related esters this isostere of acetylcholine appeared to be hydrolysed most quickly by the esterase. The spasmogenic action of acetylcarbocholine was not mentioned.

The question arises whether the acetylcholine-like action of these onium group-free isosteres of acetylcholine results from a direct cholinergic action as suggested by Burgen (1965) or whether it results from an indirect cholinergic action, either by the liberation of endogenous acetylcholine or by the protection of endogenous acetylcholine against inactivation by the inhibition of acetylcholinesterase, which would make these compounds comparable in their action to drugs like physostigmine and neostigmine.

Spasmogens acting on smooth muscle tissues such as the isolated gut can be differentiated into (*a*) directly acting cholinergic spasmogens which have an acetylcholine- or muscarine-like action based on the interaction between the drug concerned and the cholinergic receptors on the smooth muscle tissue, (*b*) indirectly acting cholinergic spasmogens, the action of

* Synthesized according to Limburg & Post (1962).

TABLE 1. INTRINSIC ACTIVITIES AND AFFINITIES OF SOME ESTERS OF CHOLINE, CARBOCHOLINE, BUTANOL AND SILICOCHOLINE

R	C–C–R (O)				Ph, OH, C–C–R (O), Ph			C_6H_{11}, OH, C–C–R (O), Ph (−)		
Rat intestine	i.a.*	pD_2	pA_2	E/O	i.a.	pA_2	E/O	i.a.	pA_2	E/O
–O–C–C–N⁺(C)(C)(C)	1·0	6·6 ± 0·12		27/8	0	8·6 ± 0·18	26/5	0	9·6 ± 0·26	19/18
–O–C–C–C(C)(C)(C)	0·65 ± 0·07	3·9 ± 0·17			0	7·5 ± 0·07	19/14	0	7·3 ± 0·17	22/21
–O–C–C–C–C	0		3·1 ± 0·15	11/8	0	7·4 ± 0·10	15/15	0	6·8 ± 0·15	23/19
–O–C–C–Si(C)(C)(C)	0·74	3·4 ± 0·11		5/2		not tested			not available	
	0		3·9	1/1						
Guinea-pig intestine	i.a.*	pD_2	pA_2	E/O	i.a.	pA_2	E/O	i.a.	pA_2	E/O
–O–C–C–C(C)(C)(C)	0·58 ± 0·18	3·9 ± 0·14		9/7 14/9		not tested			not tested	
	0		3·7 ± 0·15	18/9						
–O–C–C–C–C	0		3·3 ± 0·09	14/8	0	7·3	5/5		not tested	
–O–C–C–Si(C)(C)(C)	0·54 ± 0·10	4·0 ± 0·28		11/11 15/5	0	7·1 ± 0·13	20/5		not available	
	0		3·9 ± 0·43	8/6						

* i.a. expressed as the ratio: maximal effect of the compound/maximal effect of furtrethonium, the ± values represent S.E.
E/O: number of experiments/number of organs used in those experiments.

which is dependent on the presence of endogenous acetylcholine, probably liberated at the nerve terminals at the level of the effector system (smooth muscle). This endogenous acetylcholine then acts on the acetylcholine (muscarinic) receptors on the effector tissue, or (*c*) non-cholinergic spasmogens, in the action of which neither acetylcholine nor its receptors are involved.

These groups of spasmogens can be differentiated by testing them in the presence of different inhibitors.

Experimental

(1) Anticholinergic drugs such as atropine and lachesine in concentrations of 10^{-9}–10^{-6}M cause a parallel shift over a wide dose range of the log concentration-response curves for spasmogens acting directly on cholinergic receptors (Ariëns, 1964, 1966a, b, c; Ariëns & Simonis, 1960). For the indirectly acting cholinergic spasmogens the doses of the anticholinergic drugs mentioned cause a decline in the concentration-response curves which is occasionally preceded by a slight parallel shift. The log concentration-response curves for non-cholinergic spasmogens are not affected by the dose of 10^{-9}–10^{-6}M of atropine or lachesine. Cumulative log concentration-response curves of the spasmogens are made in the presence of different concentrations (10^{-9}–10^{-6}M) of lachesine. In the presence of a relatively high concentration of lachesine (10^{-4}M) the responses to directly-acting cholinergic drugs, in doses producing a submaximal response, and the response obtained with indirectly acting cholinergic spasmogens, are abolished. The responses to non-cholinergic spasmogens such as barium ions are unaffected.

(2) Hemicholinium (HC-3)* inhibits the synthesis of acetylcholine possibly by an inhibition of the transport of choline into the nervous elements (MacIntosh, Birks & Sastry, 1956; Long, 1961; MacIntosh, 1961). Treatment of the isolated organ with hemicholinium $1{\cdot}75 \times 10^{-3}$M, causes, after an adequate period of incubation, a large reduction in the response to the indirectly-acting cholinergic spasmogens. At $1{\cdot}75 \times 10^{-3}$M, hemicholinium has practically no influence on the response to directly-acting cholinergic and non-cholinergic spasmogens. The spasmogens are tested after incubation of the organ for 15 min with hemicholinium and with it still in the organ bath.

The inhibitive effect of hemicholinium can be counteracted to some extent by choline. If sufficient choline is available, the acetylcholine synthesis goes on in the presence of hemicholinium. Choline, 5×10^{-2}M, is applied simultaneously with hemicholinium and the spasmogens are tested after an incubation of the organ for 15 min and with the hemicholinium and choline still in the bathing fluid.

The inhibition of the response to a spasmogen by hemicholinium and the counteraction thereof by choline are strong arguments for the involvement of endogenous acetylcholine in the action of the spasmogen and therefore its classification as an indirectly-acting cholinergic spasmogen.

* Synthesized according to Long & Schueler (1954).

(3) The action of procaine on isolated organs results in the reduction of the output of acetylcholine at the nerve terminals (Feldberg & Lin, 1949; Harry, 1962). It blocks the propagation of action-potentials along the nerve fibres. In a concentration of 3×10^{-3}M, procaine blocks the responses to the indirectly-acting cholinergic spasmogens. In the concentration mentioned it leaves the responses to directly-acting cholinergic and noncholinergic spasmogens practically unaffected. The spasmogens are tested after incubation of the organ for 40 min with procaine, 3×10^{-3}M, and subsequent washing of the organ 3 times in succession with fresh Tyrode solution, which takes about 2 min.

(4) Ganglionic blocking agents competitively block the response to compounds with a ganglionic stimulant, nicotine-like action. The responses to drugs acting on receptors different from those for nicotine are practically unaffected. The spasmogens are tested in the presence of the ganglionic blocking drug hexamethonium in a concentration of 3×10^{-4}M without any pre-incubation.

Results

The anticholinergic drug lachesine effects a parallel shift in the log concentration-response curves of the directly-acting cholinergic spasmogen furtrethonium, but a depression of the curves for acetylsilicocholine as shown in Fig. 1a and b. The same is found for acetylcarbocholine. The

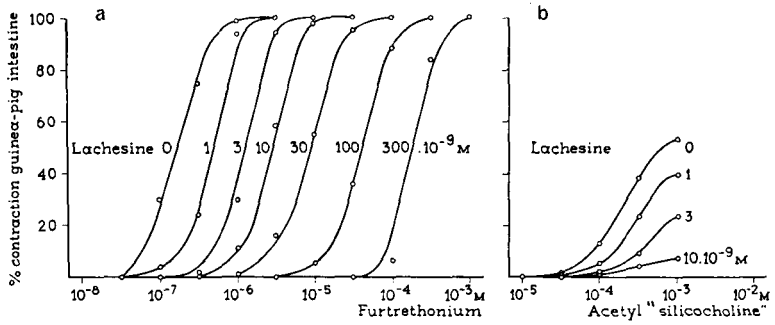


FIG. 1a. Cumulative log concentration-response curve for furtrethonium in the presence of increasing concentrations of the anticholinergic lachesine (benzilyl-*N*-dimethyl-*N*-ethylethanolamine). Note the parallel displacement of the curves indicating the competitive relation between furtrethonium and lachesine. b. Cumulative log concentration-response curve for acetylsilicocholine in the presence of increasing concentrations of lachesine (benzilyl-*N*-dimethyl-*N*-ethyl-ethanolamine). Note the depression in the curves, caused by the anticholinergic indicating an indirect cholinergic spasmogenic action for acetylsilicocholine.

depression of the log concentration-response curves for these spasmogens with relatively low concentrations of the anticholinergic indicates an indirect cholinergic action.

The contractions obtained with the various spasmogens applied as a single dose in combination with the various inhibitors are presented in

TABLE 2. DIFFERENTIATION OF VARIOUS TYPES OF SPASMOGENS

	Furtrethonium		Acetyl-"carbocholine"		Acetyl-"silicocholine"		"Acetylglycol"		Nicotine		$BaCl_2$	
In the presence of lachesine	0	(6)	0	(8)	1	(6)	2	(12)	1	(7)	128	(7)
After hemicholinium incubation	76	(9)	2	(8)	1	(9)	0	(8)	2	(9)	68	(10)
After procaine treatment	95	(9)	7	(7)	3	(12)	0	(12)	6	(6)	116	(9)
In the presence of hexamethonium	98	(6)	106	(15)	73	(20)	77	(12)	2	(19)	101	(8)

The contractions of the various spasmogens are expressed as mean percentage of the contractions under control conditions. The number of experiments is given in brackets. For further details see text.

Table 2. The contractions are expressed as percentages of the corresponding contractions in the control conditions, i.e. in the absence of blocking agents.

The doses of the spasmogens were respectively: furtrethonium 3×10^{-5}M, acetylcarbocholine 10^{-3}M, acetylsilicocholine 10^{-3}M, acetylglycol 10^{-2}M, nicotine 3×10^{-5}M, $BaCl_2$ 10^{-3}M. These doses just cause a maximal contraction of the guinea-pig ileum. Because of the desensitization phenomenon observed after furtrethonium, the application of

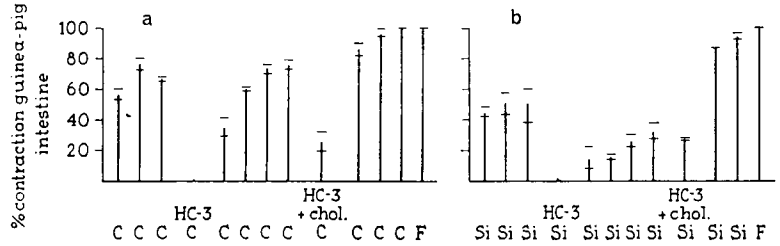


FIG. 2. The responses of the guinea-pig intestine to a single dose of acetylcarbocholine (C) (1×10^{-3}M) (a) and of acetylsilicocholine (Si) (1×10^{-3}M) (b) expressed in % of the responses to a single dose of furtrethonium (F) (3×10^{-5}M) and the inhibition thereof by incubation of the organ (15 min) with hemicholinium (HC-3) ($1{\cdot}75 \times 10^{-3}$M), as well as the antagonistic action of choline (5×10^{-2}M) with respect to the inhibitive action of hemicholinium. Note that the effects of acetylcarbocholine and acetylsilicocholine are abolished during the incubation with hemicholinium. This is in contrast to the effect of furtrethonium (see Table 2). During incubation with hemicholinium and choline the response to acetylcarbocholine and acetylsilicocholine are partially maintained. After removal of the hemicholinium the response is even enhanced. The interval between the various doses of spasmogen is 5 min, with the exception of the interval (15 min) preceding the application of the spasmogen in the presence of hemicholinium.

other spasmogens shortly after furtrethonium was avoided. When tested in the presence of lachesine (10^{-4}M), the responses of the ileum to all spasmogens except barium ions were abolished. This implies that barium chloride has to be considered mainly as a non-cholinergic directly acting spasmogen.

After 15 min incubation with hemicholinium ($1{\cdot}75 \times 10^{-3}$M) the actions of all spasmogens were inhibited with the exception of the directly-acting

cholinergic furtrethonium and the non-cholinergic spasmogen barium chloride. The partial reduction in the response to barium ions may, however, be ascribed to an indirect cholinergic component in its action. The inhibiting action of hemicholinium on acetylcarbocholine and acetylsilicocholine could be counteracted by choline (5×10^{-2}M) (Fig. 2). These experiments with hemicholinium and with the combination of hemicholinium and choline are a strong argument in favour of the involvement of acetylcholine in the spasmogenic actions of acetylcarbocholine, acetylsilicocholine and nicotine.

The influence of 40 min incubation of the ileum with procaine (3×10^{-3}M) is seen as a depression of the effect of all spasmogens used except furtrethonium and Ba^{++}.

The influence of hexamethonium (3×10^{-4}M) on the effect of the various spasmogens is seen as an inhibition of the effect of nicotine; the effects of the other spasmogens remained practically unchanged.

Discussion

These results strongly indicate that acetylcarbocholine and acetylsilicocholine are to be considered as indirectly acting cholinergic, non-nicotine-like compounds, their action being based on the stimulation of the release of acetylcholine from the presynaptic nerve terminals, probably induced at the level of these nerve terminals. Although nicotine-like compounds also act by way of the liberation of acetylcholine, this liberation is not induced at the level of the nerve terminals but at the level of the ganglionic synapse and therefore can be antagonized by hexamethonium. Besides the onium-free drugs mentioned, other compounds have been reported to have an indirect, non-nicotine-like cholinergic action (Levy & Michel-Ber, 1956; Takagi, Takayanagi & others, 1960; Carlyle, 1963; Koelle, 1963; Kosterlitz & Lees, 1963; Takagi & Takayanagi, 1966a, b). Of special importance in this respect are (*a*) esters of phenol with acids such as acetic or propionic acid, and (*b*) various acetic acid esters of alcohol, e.g. propanol, butanol, pentanol and 3-methylbutanol, which Takagi & Takayanagi (1966a) reported to act as indirect cholinergic spasmogens. In our experiments on the intestine of the rat the acetic acid esters of butanol and 3-methylbutanol had little or no spasmogenic action but mainly acted as weak anticholinergics (Ariëns, 1965). The question arises whether cholinergic receptors—other than muscarinic or nicotinic—are involved in the non-nicotinic indirect cholinergic action of the onium-free compounds studied. The fact that the central carbon and silicon atom have a certain positive charge (δ^+) might suggest a chemical relationship with acetylcholine and therefore a relation to acetylcholine receptors (Burgen, 1965). It was found, however, that the ester in which the onium group of acetylcholine was substituted by an OH-group, i.e. acetyl glycol half-ester ($MeCOOCH_2CH_2OH$) also acted as a spasmogen. On the isolated gut of the guinea-pig a pD_2 value of 2·2 was found while the maximal response obtained in a cumulative concentration-response curve was 60–100% of that of furtrethonium. In the present investigation

acetylglycol behaved in the same way as acetylcarbocholine and acetylsilicocholine (Table 2). Consequently it too may be considered as a non-nicotinic indirect cholinergic spasmogen. The hydroxy group in the ester, however, has no positive charge but has some polarity. This implies that a positive charge as present in acetylcarbocholine and acetylsilicocholine is not essential for their action. The indirect cholinergic spasmogenic action of esters such as n-pentyl acetate and n-propyl acetate reported by Takagi & Takayanagi (1966a) can also be regarded as evidence in this direction.

Acetylcarbocholine and acetylsilicocholine are related to the nitrogen free anticholinergic compounds in Table 1. Elimination of the rings from the acyl moiety in the more potent nitrogen-free anticholinergic compounds, as expected, results in a strong decrease in their activity, seen as a decrease in the pA_2 values. The question arises whether besides having an indirect cholinergic action, these nitrogen-free compounds still have some anticholinergic action. An anticholinergic activity if present in acetylcarbocholine and acetylsilicocholine may manifest itself if tested against a directly-acting cholinergic drug such as furtrethonium on organs kept in the ice box at 2° for 24 hr, as these are practically irresponsive to the indirectly-acting spasmogens. As demonstrated in Figure 3a,

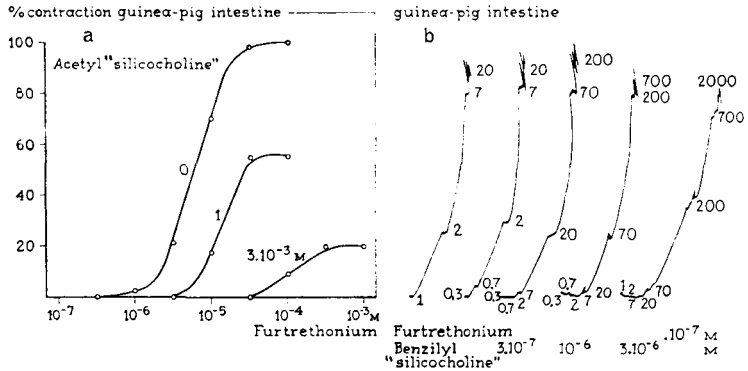


FIG. 3.a. Cumulative log concentration-response curve for furtrethonium, tested in the presence of increasing concentrations of acetylsilicocholine obtained on a piece of isolated guinea-pig ileum (stored in the ice-box for 24 hr). Note that on the gut, so pretreated, acetylsilicocholine has no spasmogenic action of its own, but behaves as a non-competitive (the depression in the curves) and a competitive (the parallel displacement of the curves) antagonist of furtrethonium. b. Registrogram for cumulative dose-response curves, obtained with furtrethonium in the presence of various concentrations of benzilylsilicocholine. Note that the parallel shift in the curves indicates an anticholinergic action of benzilylsilicocholine. The depression in the curves, which becomes more manifest if higher doses of benzilysilicocholine are used, indicates a non-competitive spasmolytic action for this compound.

acetylsilicocholine antagonizes furtrethonium. There is a decline in the log concentration-response curves indicating a non-competitive, papaverine-like spasmolytic action which is combined with a parallel shift in the curves. This indicates the anticholinergic component in the spasmolytic action of acetylsilicocholine. Analogous results were obtained with

acetylcarbocholine in concentrations of 10^{-3}M and other acetyl esters of simple alcohols like 3-methylbutanol and butanol. A schematic representation of the mode of action of acetylcarbocholine and acetylsilicocholine is given in Fig. 4. On the receptor system R_{II}, acetylcarbocholine and acetylsilicocholine, because of their anticholinergic action, have an action as competitive antagonists of the acetylcholine liberated. On the receptor system R'_{II}, a noncompetitive, "papaverine-like" spasmolytic action is induced by acetylcarbocholine and acetylsilicocholine. One of the consequences of this multiplicity in actions is that the spasmogenic action induced on the receptor system R_I, by virtue of the liberation of endogenous acetylcholine acting on the receptor system R_{II}, is inhibited because of the inhibitory actions on the receptor systems R_{II} and R'_{II}. The result is that in the cumulative log concentration-response curves of acetylcarbocholine and acetylsilicocholine an auto-inhibition becomes manifest. The hemiacetate of glycol, "acetyl-glycol" is devoid of anticholinergic properties in concentrations up to $3 \cdot 10^{-2}$M.

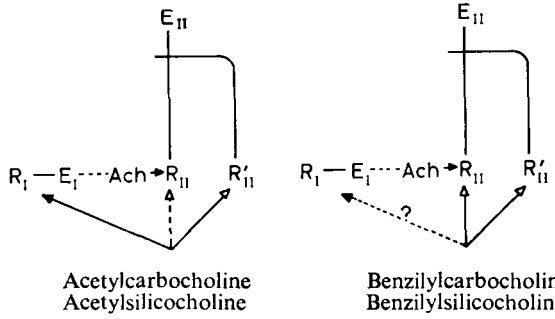


Fig. 4. Probable sites of action of carbocholine and silicocholine esters. (Solid arrows stimulant action; open arrows inhibiting action). R_I are the receptors located at the nerve terminal, E_I is the effector system on which the liberation of endogenous acetylcholine is induced. R_{II} are the cholinergic (muscarinic) receptors on which the acetylcholine liberated induces its spasmogenic effect. The smooth muscle fibres represented by E_{II} serve as the final effector system. R'_{II} represents the receptors on which a non-competitive spasmolytic action is induced.

The elimination of the aromatic rings and the hydroxy-group from the acidic moiety in benzilylcarbocholine and related compounds results in a strong decrease in the anticholinergic activity (Ariëns, 1965, 1966a, b) (Table 1). As shown in Fig. 3a, acetylsilicocholine has weak anticholinergic properties. Introduction of aromatic rings and an hydroxy-group in the acidic moiety, leading to benzilylsilicocholine, results in an increase in the anticholinergic activity again (Fig. 3b). Benzilylsilicocholine*, tested on the isolated gut of the guinea-pig, has a relatively high anticholinergic activity (pA_2 7·1). On the isolated heart of the frog both benzilylcarbocholine and benzilylsilicocholine showed a clear-cut anticholinergic

* Obtained by coupling trimethylsilylethanol (silicocholine) with diphenylchloroacetylchloride followed by hydrolysis to eliminate the chloro-groups.

action. A schematic representation of the mode of action of benzilylcarbocholine and benzilylsilicocholine is given in Fig. 4. These compounds act primarily as competitive antagonists of acetylcholine on the cholinergic (muscarinic) receptors R_{II}. In higher concentrations they also have a non-competitive, papaverine-like spasmolytic action induced on the receptors R'_{II}. Whether an indirect cholinergic action is also present remains unanswered. The blockade of the receptor system R_{II} at relatively low concentrations of these esters may interfere with the effectuation of such an action.

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References

Ariëns, E. J. (1964). *Molecular Pharmacology*, Vol. I, New York: Academic Press.
Ariëns, E. J. (1965). Scientific and Technical Symposium. 112th Ann. Meeting American Pharmac. Ass., March 28–April 2, Detroit: Michigan.
Ariëns, E. J. (1966a). In *Advances in Drug Research*, Vol. 3, editors: Harper, N. J. & Simmonds, A. B., pp. 235–285. London: Academic Press.
Ariëns, E. J. (1966b). *Proc. Meeting New York Academy of Sciences on Cholinergic Mechanisms* (May 1966), in the press.
Ariëns, E. J. (1966c). *Arzneimittel-Forsch.*, **16**, 1376–1393.
Ariëns, E. J. & Simonis, A. M. (1960). *Archs int. Pharmacodyn. Thér.*, **127**, 479–495.
Ariëns, E. J. & Simonis, A. M. (1964). *J. Pharm. Pharmac.*, **16**, 137–157.
Burgen, A. S. V. (1965). *Br. J. Pharmac. Chemother.*, **25**, 4–17.
Carlyle, R. F. (1963). *Ibid.*, **21**, 137–149.
Ellenbroek, B. W. J. (1964). Thesis, University Nijmegen, The Netherlands.
Feldberg, W. & Lin, R. C. Y. (1949). *Br. J. Pharmac. Chemother.*, **4**, 33–94.
Funcke, A. B. H., Rekker, R. F., Ernsting, M. J., Tersteege, H. M. & Nauta, W. Th. (1959). *Arzneimittel-Forsch.*, **9**, 573.
Funcke, A. B. H., Rekker, R. F., Ernsting, M. J., Tersteege, H. M. & Nauta, W. Th. (1960). *Ibid.*, **10**, 767.
Harry, J. (1962). *Br. J. Pharmac. Chemother.*, **19**, 42.
Koelle, G. B. (1963). *Handb. exp. Pharmak.* 15, p. 264. Berlin: Springer-Verlag.
Kosterlitz, H. W. & Lees, G. M. (1964). *Pharmac. Rev.*, **16**, 301–339.
Levy, J. & Michel-Ber, E. (1956). *Actualités Pharmacologiques*, p. 35–81, Vol. 9. Paris: Masson et Cie.
Limburg, W. W. & Post, H. W. (1962). *Recueil*, **81**, 430.
Long, J. P. & Schueler, F. W. (1954). *J. Am. pharm. Ass., Sci. Edn*, **43**, 79–86.
Long, J. P. (1961). *J. mednl pharm. Chem.*, **4**, 505–510.
MacIntosh, F. C., Birks, R. I. & Sastry, P. B. (1956). *Nature, Lond.*, **178**, 1181.
MacIntosh, F. C. (1961). *Fedn Proc. Fedn Am. Socs exp. Biol.*, **20**, 562.
Takagi, K., Takayanagi, I., Ishida, Y. & Moritoki, H. (1965). *Archs int. Pharmacodyn. Thér.*, **158**, 354–359.
Takagi, K. & Takayanagi, I. (1966a). *Jap. J. Pharmac.*, **16**, 211–216.
Takagi, K. & Takayanagi, I. (1966b). *J. Pharm. Pharmac.*, **18**, 795–800.
Whittaker, V. P. (1954). *Progress in Stereochemistry*, Vol. 1, 285. New York: Academic Press.