Br. J. Pharmac. Chemother. (1966), 27, 185–195.

THE ACTION OF TERTIARY AND QUATERNARY ARECAIDINE AND DIHYDROARECAIDINE ESTERS ON THE GUINEA PIG ISOLATED ILEUM

BY

HOLGER GLOGE, HEINZ LÜLLMANN AND ERICH MUTSCHLER*

From the Institute of Pharmacology, Christian-Albrechts-University, Kiel, W. Germany

(*Received March* 3, 1966)

Arecaidine, a constituent of the betel nut, exerts a stimulating action on cholinergically innervated structures (Marmé, 1890; Plesch, 1895; Dixon, 1924; Stefansson, 1937; von Euler & Domeij, 1945; Holtz & Westermann, 1955; van Rossum, 1962). It therefore seemed of interest to modify the molecule of arecoline—i.e., arecaidine methyl ester—systematically and to investigate the pharmacological properties of the new compounds, which were synthesized by Mutschler (1964). The results might give us a better general picture of the interaction between drugs and cholinoceptive receptors. This paper deals with the investigation of a number of arecaidine derivatives on the isolated ileum of the guinea-pig. The results suggested that the action of these compounds is dependent on the length of the side-chain, on hydrogenation of the double bond in the ring and on quaternization of the ring nitrogen atom. Formulae of the compounds investigated are represented in Table 3.

METHODS

The experiments were carried out on the isolated ileum of the guinea-pig as described by Magnus (1904). The Tyrode solution was composed as follows: 8.0 g sodium chloride; 0.9 g potassium chloride; 0.2 g. calcium chloride; 0.1 g magnesium chloride; 0.25 g sodium dihydrogen phosphate; 1.0 g sodium bicarbonate; 1.0 g glucose; water to 1,000 ml. Oxygen was bubbled continuously through the solution, which was kept at 30° C. At this temperature the *p*H of the solution was 8.0. Each piece of ileum was stimulated by means of the selected agonist at intervals of 5 min. When after a maximal effect desensitization occurred, we waited until a test dose of acetylcholine again provoked the same contraction response as observed before the desensitization phenomenon. The latter especially took place after maximal responses provoked by acetylcholine, arecoline or arecaidine ethyl ester.

The effects of all compounds investigated were compared with the response to arecoline and correlated with the dose-response curve of the latter drug. The sensitivity of the isolated ileum towards arecoline remained constant for several hr, as demonstrated in control experiments. The dose-response curves were calculated by the method of Ariens (1964) and characterized by means of the following parameters: 1, *intrinsic activity*, with respect to the maximal contraction amplitude observed after arecoline administration; 2, *affinity*—i.e., the reciprocal value of the drug concentration which caused 50% of the maximal effect (ED50); and 3, *slope* of the straight lines which

* Pharmaceutical Institute, University of Mainz, W. Germany.

are obtained upon transformation of the sigmoid-shaped dose-response curve, as described by Ariens (1964). Moreover, a further characterization of the cholinomimetic compounds was obtained upon investigation to which extent their agonistic action could be blocked by atropine. For the quantitative evaluation of a possible antagonistic action the method described by Schild (1949) was used. Atropine was added to the bath fluids in the concentrations 3×10^{-10} and 10^{-9}M, respectively.

Some of the esters of arecaidine or dihydroarecaidine had no intrinsic activity. The affinity of these compounds for the receptors was investigated upon evaluation of their influence on the effects of acetylcholine. For this purpose, the ileum was stimulated regularly, at intervals of 5 min, by means of a constant concentration of acetylcholine, approximately corresponding to the ED50. Before every second or third effect the antagonist to be investigated was added to the bath fluid in increasing concentrations (Fig. 3). The extent to which the effect of acetylcholine could be inhibited by the antagonist was also evaluated by means of a dose-response curve and described quantitatively.

RESULTS

1. *Comparison of arecoline with acetylcholine and its tertiary analogue, dimethylaminoethyl acetate*

For arecoline—i.e., arecaidine methyl ester—40 dose-response curves were obtained. The mean value for ED50 proved to be 5.8×10^{-8}M (confidence limits from 5.0 to 6.7

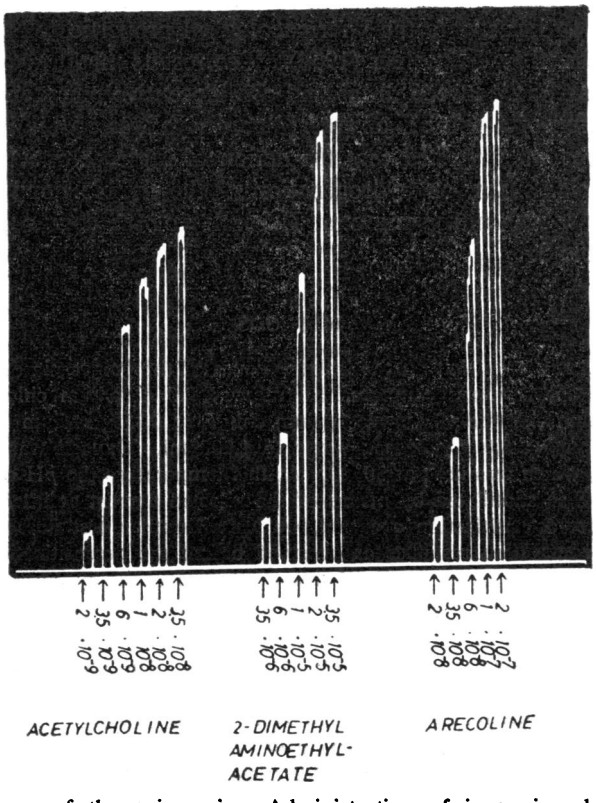


Fig. 1. Isolated ileum of the guinea-pig. Administration of increasing doses of acetylcholine, 2-dimethylaminoethyl acetate and arecoline. Concentrations in mol/l. Administration of the agonists every 5 min.

for $P=0.05$). The slope of the dose-response curve, after transformation according to Ariens (1964), was 61°. In Fig. 1 the original dose-response curves for acetylcholine, arecoline and dimethylaminoethyl acetate are represented. Considerable differences in the sizes of the maximally possible amplitudes of contraction after arecoline, acetylcholine and dimethylaminoethyl acetate were noted. When the intrinsic activity of arecoline was arbitrarily set equal to 1.0, that of acetylcholine was only 0.80 ± 0.03 (mean $\pm$ S.E.M.), whereas that of dimethylaminoethyl acetate proved as high as the intrinsic activity of arecoline.

The slope of the dose-response curves was identical for all the above three compounds. The affinities on the other hand showed considerable differences, as may be concluded from the magnitude of the concentrations necessary to obtain 50% of the maximal contractions (Table 1).

Since enzymatic degradation of acetylcholine might be responsible for the apparently reduced intrinsic activity, the experiments were repeated in presence of the anticholinesterase compound physostigmine. As might be expected, the dose-response curve in the presence of physostigmine showed a shift to the left. Again, the intrinsic activity ($0:85 \pm 0.07$) was lower than that of arecoline. Addition of ganglionic blocking agents like trimetaphan or hexamethonium to the bath fluid did not cause any increase of the contraction amplitude.

2. Action of arecaidine esters

The stimulating action of the arecaidine esters proved dependent on the length of their side-chain (Fig. 2). The dose-response curves for these esters are represented in

TABLE 1

AFFINITY, INTRINSIC ACTIVITY AND SLOPE OF THE DOSE-RESPONSE CURVE OF TERTIARY ARECAIDINE AND DIHYDROARECAIDINE ESTERS, AND OF ACETYLCHOLINE AND ITS TERTIARY ANALOGUE DIMETHYLAMINOETHYL ACETATE

Experiments performed on the isolated ileum of the guinea-pig. Relative values based on arecoline as standard compounds * no quantitative evaluation possible † partial antagonist ‡ slope of the dose-response curve as inhibitor

Compound	rel. affinity	Intrinsic activ.	rel. rise of the dose-response curve
Acetylcholine	10	0·80	1·0
Dimethylamino-ethyl acetate	0·004	1·00	1·0
Arecoline (Methyl ester)	1 (=5, 8×10^{-6}M)	1 (=max. contraction)	1 (=61°)
Arecaidine ester			
Ethyl-	3·5	1·00	1·0
n-Propyl-	0·03	0·54	0·6
iso-Propyl-	0·006	0·36	0·45
n-Butyl-	0·07	0·90	0·85
iso-Butyl-	0·02	0·47	0·55
Amyl-	0·05	0·65	0·7
Dihydroarecaidine ester			
Methyl	0·004	0·93	0·95
Ethyl-	0·002	0·65	0·65
n-Propyl-	—	0·27†	— *
	0·002	—	0·9
iso-Propyl-	0·004	— †	0 9 ‡
n-Butyl-	0·002	—	1·0 ‡
iso-Butyl	0·003	—	0·85‡

Fig. 3. The methyl and ethyl esters possessed the highest intrinsic activity. Like acetylcholine, esters with a longer side-chain could not produce a maximal effect. The affinity of the arecaidine ethyl ester was approximately 3.5 times higher than that of arecoline, though but one third of that of acetylcholine. Hence the ethyl ester is a very active compound, its ED50 amounting to approximately 1.5×10^{-8}M. The transition of the ethyl ester to the corresponding n-propyl compound was accompanied by a considerable loss of activity. The affinity decreased by a factor 100, whereas the intrinsic activity and the slope of the dose-response curve were both reduced by about 50%. The n-butyl and n-amyl esters were somewhat more active than the n-propyl ester, the iso-propyl and iso-butyl esters on the other hand proved still less active than the n-propyl ester. The parameters in question are listed in Table 1.

3. Action of the dihydroarecaidine esters

Hydrogenation of the double bond in the ring considerably decreased the stimulating action of the arecaidine esters. As for the methyl ester, the affinity decreased by a factor 250 upon hydrogenation. For the ethyl ester a decrease by a factor 1,000 was observed. The intrinsic activity of the dihydroarecaidine esters diminished dependent upon the length of their side-chains. The intrinsic activity of the n-propyl ester, for instance, was

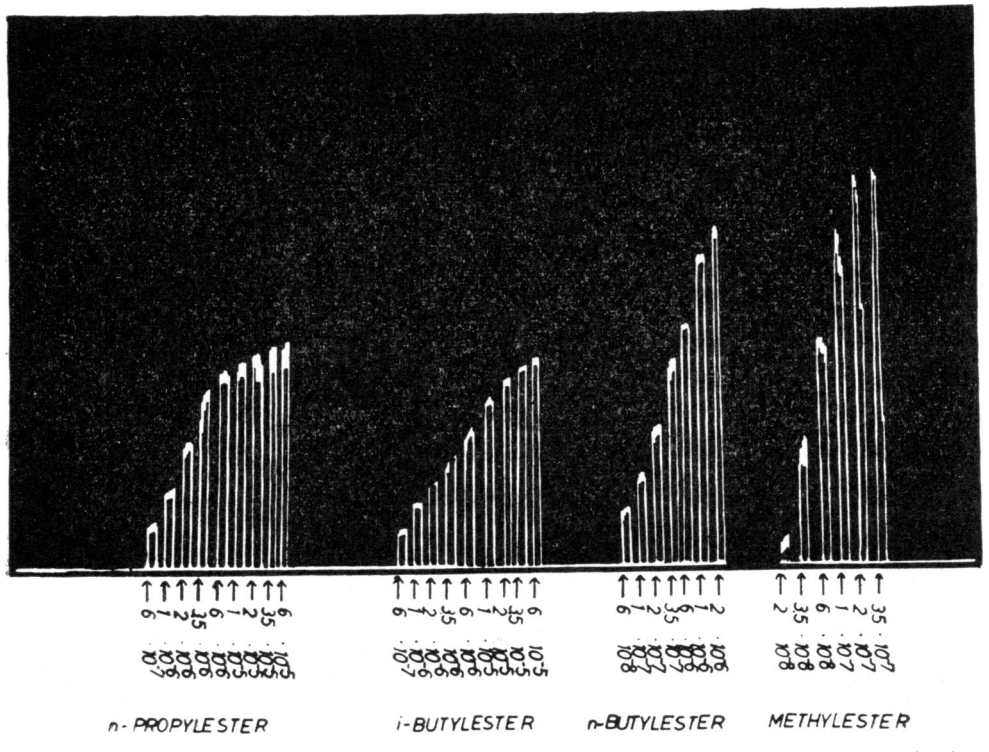


Fig. 2. Isolated ileum of the guinea-pig. Administration of increasing doses of n-propyl, iso-butyl, n-butyl and methyl ester, respectively. Concentrations in mol/l. Administration of the agonists every 5 min.

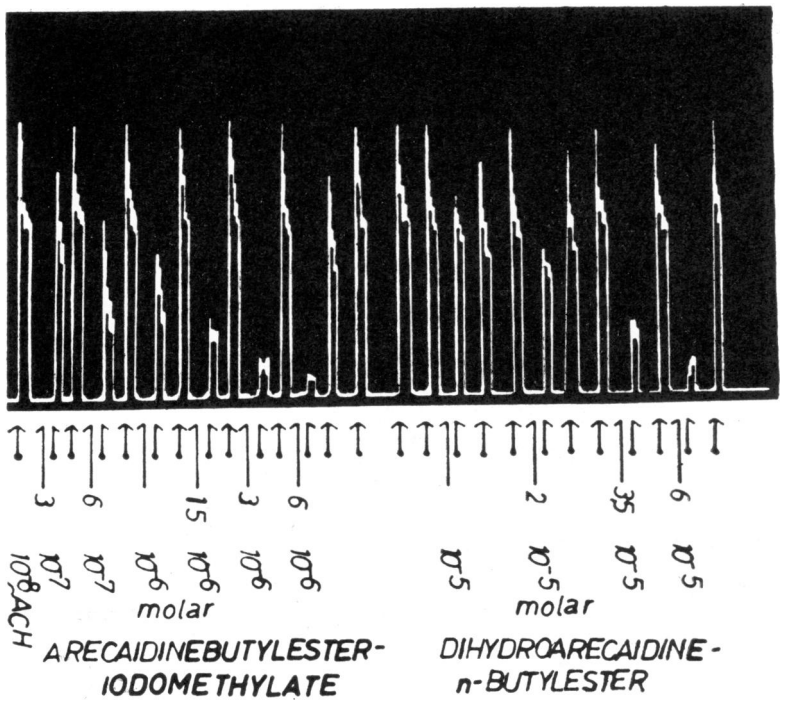


Fig. 3. Isolated ileum of the guinea-pig. Administration of constant doses of acetylcholine. Before every second or third effect an inhibitor is given in increasing concentrations. Concentrations of the agonist acetylcholine and of the antagonists arecaidine butyl ester iodomethylate and dihydroarecaidine butyl ester in mol/l. Agonists given every 5 min ; the antagonist is administered 1 min before the agonist.

but 0.27. This compound, however, also showed inhibiting properties and might thus be qualified as a partial antagonist (Ariens, 1964).

The isopropyl-, n-butyl and isobutyl esters of dihydroarecaidine were inhibitors. They were able to abolish the stimulating action of acetylcholine and other agonists. The various parameters are enumerated in Table 1.

4. Action of the quaternary arecaidine esters

Iodomethylation of arecoline led to a loss of affinity by a factor 10, whereas intrinsic activity remained almost unaffected. A far greater loss in affinity was observed upon quaternization of arecoline as the corresponding ethiodide or propiodide. As shown in Table 2, the intrinsic activity of the latter two quaternary compounds is considerably lower than that of the free base.

The methiodide of arecaidine ethyl ester behaved like a partial antagonist. The corresponding ethiodide, however, turned out to be a genuine inhibitor. All esters of arecaidine methiodide with longer side-chains acted as pronounced inhibitors. This inhibitory activity may be qualified as atropine-like, since only the stimulating actions of acetylcholine and pilocarpine were antagonized. The effects of histamine, serotonin and Ba^{++} ions in the concentrations given in Table 2 remained unchanged.

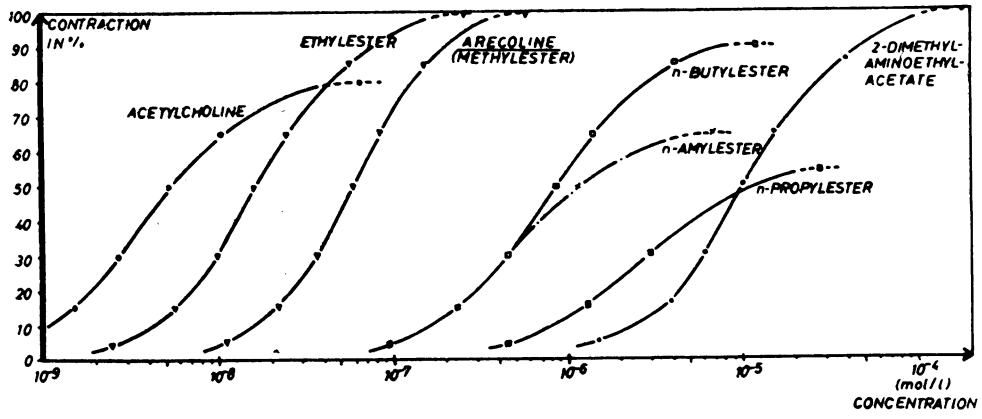


Fig. 4. Concentration-activity curves for acetylcholine, dimethylaminoethyl acetate and the indicated arecaidine esters. Experiments on the isolated ileum of the guinea-pig. Abscissa: log concentration (mol/l). Ordinate: % of the maximal contraction amplitude after arecoline. In every experiment arecoline (n = 40) was the standard compound. The curves have been calculated from the various individual curves according to Ariens (1964).

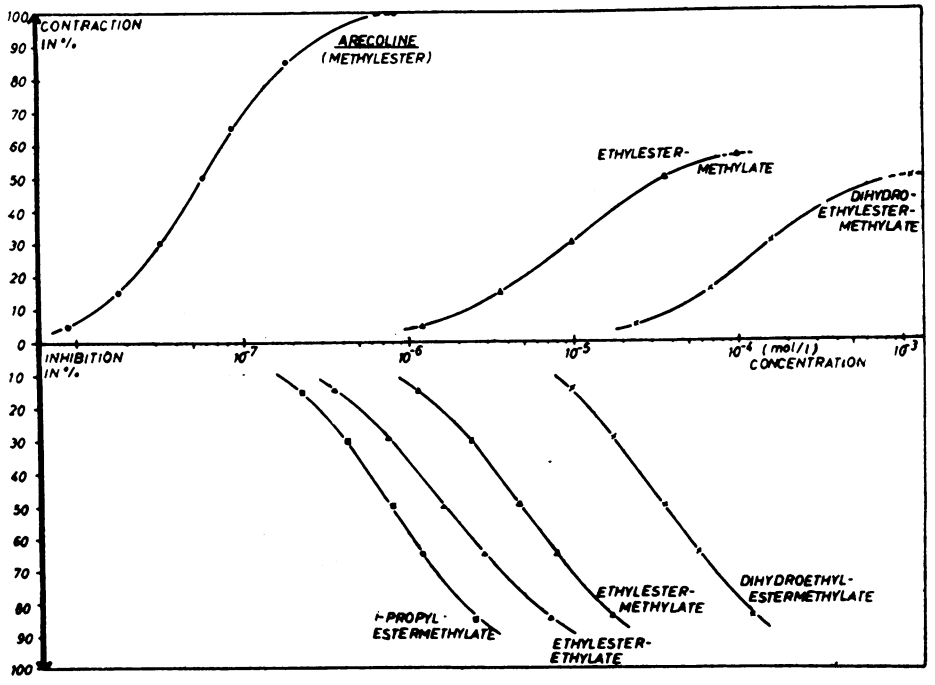


Fig. 5. Concentration-activity curves for arecaidine and dihydroarecaidine derivatives. Experiments on the isolated ileum of the guinea-pig. Abscissa: log concentration (mol/l) of the esters. Ordinate: upper part: % of maximal contraction amplitude after arecoline; lower part: % inhibition of the acetylcholine effect. As in Fig. 4 arecoline was the standard compound. The methiodides of arecaidine—and dihydroarecaidine ethyl esters are partial antagonists. Arecaidine isopropyl methiodide and the ethiodide of arecaidine ester are mere inhibitors.

The esters of the quaternary dihydroarecaidine esters behaved similarly to the unsaturated parent compounds. The affinity, however, was reduced by a factor 10 upon hydrogenation. The methiodide of dihydroarecaidine methyl ester showed cholinergic properties. Again, the corresponding ethyl ester proved a partial antagonist. Extension of the side-chain yielded only inhibitors.

5. Inhibition of the agonists by atropine.

As described under Methods, the inhibitory properties of atropine on the agonists were investigated according to Schild (1949). For the various drugs the x-values at two different atropine concentrations were determined. Acetylcholine was used as standard compound. In order to produce the same contraction response as obtained without any inhibitor, approximately a 25-fold dose of acetylcholine had to be administered in the presence of an atropine concentration of 10^{-9}M. For a few other agonists unusual values were obtained. Only the latter compounds will be dealt with here. In the presence of atropine 10-, 14- and 18-fold doses of respectively arecoline, dimethylaminoethyl acetate and arecaidine ethyl ester had to be administered in order to reach the initial level of the contraction amplitude. Hence the blockade of the acetylcholine receptors by atropine was more easily broken by the above-mentioned three agonists than by acetylcholine itself. These three agonists also had a higher intrinsic activity than acetylcholine. The stimulating action of the various arecaidine esters could not be

TABLE 2

AFFINITY, INTRINSIC ACTIVITY AND RISE OF THE DOSE-RESPONSE CURVES OF QUATERNARY ARECAIDINE AND DIHYDROARECAIDINE ESTERS

Experiments on the guinea-pig isolated ileum. Relative values based on arecoline as standard compound
* partial antagonist † has nicotine-like action

Compound	quaternized as	rel. affinity	Intrins. act.	rel. rise of the dose-response curve
Arecoline	—	1 (=5×10^{-8}M)	1 (=max. contraction)	1 (=61°)
Arecaidine ester				
Methyl-		0·12	0·93	0·9
Ethyl-†		0·004	0·57*	0·65
		0·01	—*	0·85
Propyl-	methiodide	0·02	—	0·9
iso-Propyl		0·07	—	0·95
n-Butyl-		0·02	—	0·9
iso-Butyl		0·02	—	1·0
Methyl-		0·003	0·74	0·65
Ethyl-	ethiodide	0·04	—	0·85
Methyl-	propiodide	0·0002	0·63	0·8
Dihydroarecaidine ester				
Methyl-		0·01	0·98	0·95
Ethyl-		0·0005	0·50	0·7
		0·002	—*	0·85
n-Propyl-	methiodide	0·003	—	0·9
iso-Propyl-		0·007	—	0·9
n-Butyl-		0·007	—	1·0
iso-Butyl-		0·005	—	0·9

blocked by hexamehonium. Nevertheless, there was one exception—i.e., the methiodide of arecaidine ethyl ester. Its action as well as that of nicotine could be abolished by ganglionic blocking agents (Kummer, Lüllmann & Mutschler, 1966).

DISCUSSION

Like muscarine, the tertiary arecaidine esters exert a stimulating action on the smooth muscle of the guinea-pig ileum. The affinity and intrinsic activity are to a considerable extent dependent on the length of the side-chains of the esters (Table 1). The ethyl ester possesses highest affinity ($ED50 = 1.5 \times 10^{-8}$M)—i.e., approximately one third of that of acetylcholine. The intrinsic activities of arecoline and arecaidine ethyl ester are significantly higher than that of acetylcholine. Surprisingly, dimethylaminoethyl acetate, the tertiary analogue of acetylcholine, also possesses a higher intrinsic activity than the latter compound, whereas its affinity is more than 1,000 times lower than that of acetylcholine. The action of dimethylaminoethyl acetate particularly depends on the *p*H of the medium, as will be dealt with in detail in a subsequent paper. The homologues following on the ethyl ester—i.e., arecaidine n-propyl- and isopropyl esters—show affinities which are 100 and 500 times lower than that of the ethyl ester, respectively. The intrinsic activity of both propyl esters is also lower. Esters with a longer side-chain are not quite as inactive as the propyl esters.

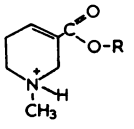
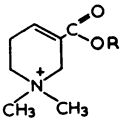
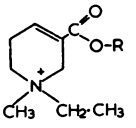
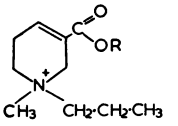
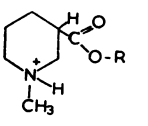
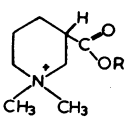
As demonstrated by investigations on the blood pressure of the cat (Kummer *et al.*, 1966) and on the guinea-pig isolated auricle the arecaidine esters possess genuine muscarine-like activity. This observation for tertiary nitrogen compounds is rather surprising, since chiefly quaternary nitrogen derivatives are known to react with acetylcholine receptors. Recently van Rossum (1962) has emphasized this phenomenon. Equally surprising is the observation that our tertiary compounds show a higher intrinsic activity than acetylcholine. Arecaidine esters with a short side-chain and also pilocarpine have a high affinity to the acetylcholine receptor. These findings suggest that a tertiary nitrogen atom in the ring is just as suitable for the formation of a bond with the receptor as are quaternary, aliphatic nitrogen atoms summarized by Ariens, 1964; and Barlow, 1964).

Hydrogenation of the double bond in the ring considerably alters the pharmacological activity of the esters investigated. The affinity decreases, whereas esters with a longer side-chain possess no more intrinsic activity. Consequently, these dihydroarecaidine esters are acting as inhibitors. Their inhibitory activity, which is not very specific, should be considered rather papaverine-like than atropine-like. Hydrogenation of the double bond in the ring leads to considerable changes in the shape of the molecule. After hydrogenation the ring either accepts the chair or boat configuration. Accordingly, the ring substituent at carbon atom 3 is either in the equatorial or axial position. Thus the steric configuration and also the distance between nitrogen atom and ester group will undergo alterations. Moreover, abolition of the conjugated double bond system alters the charge distribution. The basicity of the nitrogen atom may also be influenced. Investigations on the above chemical alterations in connection with the biological activity of the compounds thus obtained have been carried out and will be published elsewhere.

TABLE 3

SURVEY OF THE MODE OF ACTION AND THE ACTIVITY OF THE INVESTIGATED ARECAIDINE DERIVATIVES

Experiments on isolated ileum of the Guinea-pig. S = stimulating action; I = inhibitory action; † = compound has nicotine-like activity. The number of asterisks is a measure for the activity : **** ED50 in the range 10^{-8}—10^{-7}M *** ED50 in the range 10^{-7}—10^{-6}M ** ED50 in the range 10^{-6}—10^{-5}M * ED50 in the range 10^{-5}—10^{-4}M

R						
Methyl	S**** (Arecoline)	S***	S*	(S)	S*	S**
Ethyl	S****†	S* I**	I***		S*	S* I*
n-Propyl	S**	I**			S* I*	I*
iso-Propyl	S*	I***			I*	I*
n-Butyl	S**	I**			I*	I*
iso-Butyl	S**	I**			I*	I*

The affinity and the intrinsic activity of the quaternized arecoline are both dependent on the size of the substituent introduced on the nitrogen atom. Thus, in comparison wth arecoline, which possesses a tertiary, protonated nitrogen atim (Burgen, 1965) the corresponding methiodide, ethiodide and propiodide respectively show intrinsic activities which are 10, 300 and 5,000 times lower than that of arecoline itself. A still more pronounced loss of both affinity and intrinsic activity takes place upon iodomethylation of arecaidinethyl ester. The affinity is reduced by approximately a factor 1,000 and the original ester, an agonist with maximal intrinsic activity is converted into a partial antagonist. Arecaidine ethyl ester ethiodide is already an antagonist. The methiodides of arecaidine esters with a longer side-chain are inhibitors with atropine-like action. The slopes of the dose-response curves for these inhibitors are similar to that of the arecoline curve. The affinity of the most active inhibitor—i.e., the methiodide of arecaidine isopropyl ester—is but 14 times lower than that of arecoline. For the quaternized isopropyl ester the ED50 is about 3.5×10^{-7}M. As already observed for the tertiary esters, the affinity of the quaternary compounds is considerably diminished upon hydrogenation of the ring double bond. In Table 3 a simplified survey of the results is given. Esters of (tertiary) arecaidine with short side-chains possess the highest intrinsic activity. The latter is diminished upon quaternization. Whereas the intrinsic activity depends on the length of the side-chain, the affinity of the quaternary compounds is hardly influenced by this parameter. Consequently, esters with longer side-chains are inhibitors with an atropine-like action. For a high intrinsic activity of both tertiary and quaternary esters a short side-chain seems to be essential. Transformation of tertiary esters with a longer side-chain, beginning with the n-propyl esters, into the corresponding, quaternary compounds converts cholinomimetic drugs with moderate affinity into inhibitors with at least the same affinity. This phenomenon is particularly pronounced in the case of the ethyl ester. This tertiary ester, of which the ring nitrogen is protonated, shows a high intrinsic activity. Quaternization by means of a methyl group yields a partial antagonist with a nicotine-like component: the intrinsic activity has decreased considerably or has even disappeared completely with respect to the muscarine-like receptor. If an ethyl

group is bound to the nitrogen atom instead of methyl, the resulting quaternized compound shows no more intrinsic activity at all. Since the affinity decreases but very little, an inhibitor is obtained.

SUMMARY

1. A homologous series of tertiary and quaternary arecaidine esters (methyl to iso-butyl) as well as the corresponding dihydro compounds were investigated quantitatively on the isolated ileum of the guinea-pig.

2. The tertiary arecaidine esters are agonists. Highest activities are observed for the ethyl ester (ED50 $= 1.5 \times 10^{-8}$M) and for arecoline, the methyl ester (ED50 $= 5.8 \times 10^{-8}$M). Esters with a longer side-chain show considerably lower activity. The intrinsic activities of arecaidine ethyl ester, of arecoline and of dimethylaminoethyl acetate are higher than that of acetylcholine.

3. Hydrogenation of the double bond in the ring markedly reduces the affinity and intrinsic activity of the tertiary arecaidine esters. Hydrogenated esters with a longer side-chain act as inhibitors.

4. Quaternization by means of iodomethylation exerts varying influences on the intrinsic activities of arecaidine esters. In the case of the methyl ester the intrinsic activity is somewhat reduced whereas that of the ethyl ester considerably decreases upon iodomethylation, thus yielding a partial antagonist. Similar transformation of esters with a longer side-chain leads to a complete loss of intrinsic activity. The quaternized compounds thus obtained are inhibitors with atropine-like action. Iodoethylation and iodopropylation even abolish the intrinsic activities of esters with a short side-chain.

5. Hydrogenation of the double bond in the ring of the quaternary compounds similarly diminishes the activity as observed for the tertiary compounds.

6. For aliphatic nitrogen atoms quaternization is essential in order to enable a reaction with the acetylcholine receptors of the muscarine type. In the case of ring nitrogen atoms, the tertiary, protonated form is necessary for obtaining a high intrinsic activity upon combination with the receptor molecule. In arecaidine esters, quaternization of the ring nitrogen atom reduces or even destroys intrinsic activity, in proportion to the length of the ester side-chain.

We are indebted to Dr P. A. Van Zwieten for his comments and for the translation of the manuscript.

REFERENCES

ARIENS, E. J. (1964). Molecular pharmacology. Academic Press, New York and London.

BARLOW, R. B. (1964). Introduction to chemical pharmacology. Methuen and Co., London.

BURGEN, A. S. V. (1965). The role of ionic interaction at the muscarinic receptor. *Br. J. Pharmac. Chemother.* **25**, 4–17.

DIXON, W. E. (1924). Areca-Alkaloide. Handbuch der experimentellen Pharmakologie. J. SPRINGER, Volume 2, p. 813–816.

VON EULER, U. S. & DOMEIJ, B. (1955). Nicotine-like actions of Arecoline. *Acta. pharmac. tox.*, **1**, 263–269

HOLTZ, P. & WESTERMANN, E. (1955). Versuche mit Acetyl-, Propionyl- und Butyrylcholin am isolierten. Herzvorhofpräparat. *Naunyn-Schmiedebergs Arch. exp. Path. Pharmak.*, **225**, 421–427.

KUMMER, B., LÜLLMANN, H. & MUTSCHLER, E. (1966). Über die Wirkung von Arecaidin-Estern auf den Blutdruck der Katze. *Naunyn-Schmiedebergs Arch. exp. Path. Pharmak.*, in the press.

MAGNUS, R. (1904). Versuche am überlebenden Dünndarn von Säugetieren. *Pflügers Arch. Physiol.*, **102**, 123–151.

MARMÉ, W. (1890). Arecanüsse. *Ther. Mh.*, **4**, 291–294.

MUTSCHLER, E. (1964). Vergleichende pharmakologische Untersuchungen von Derivaten des Damascenins und Arecolins. Habilitationsschrift, Mainz 1964.

PLESCH, J. W. (1895). Beitrag zur Kenntnis der Wirkung von Arecolin. Thesis, Kiel.

VAN ROSSUM, J. M. (1962). Pharmacodynamics of parasympathetic drugs. Mechanism of action and structure-action relationship of tertiary ammonium salts. *Archs. int. Pharmacodyn. Ther.* **140**, 592–605.

SCHILD, H. O. (1949). pA_x and competitive drug antagonism. *Br. J. Pharmac. Chemother.*, **4**, 277–280.

STEFANSSON, K. (1937). Die Darmwirkung des Arecolin. *Naunyn-Schmiedebergs Arch. exp. Path. Pharmac.* **185**, 429–434.