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Pyrrolizidine alkaloids: actions on muscarinic receptors in the guinea-pig ileum

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Summary

1. Eleven pyrrolizidine alkaloids have been tested on the isolated guinea-pig ileum preparation.

2. Platyphylline, supinine, heleurine and cynaustraline were more potent in antagonizing responses to acetylcholine and carbachol than responses to histamine. Their anticholinergic activity appeared to involve a competitive mechanism.

3. Lasiocarpine, monocrotaline, spectabiline, sarracine, 7-angelylheliotridine, heliotrine and senecionine had similar antagonistic potencies against responses to both acetylcholine and histamine.

4. The alkaloids had no appreciable activity as antagonists of acetylcholine in the isolated toad rectus abdominis preparation.

5. These results are discussed with respect to interactions of the alkaloids at receptor sites involved in anticholinergic activity at the muscarinic receptor.

Introduction

Numerous plant families of world wide distribution contain pyrrolizidine alkaloids. The alkaloids occur particularly in the genera *Senecio, Crotalaria* and *Heliotropium.* Most are esters, and in those used in the present work esterification occurs with hydroxy groups in the C7 position of the pyrrolizidine ring and/or with hydroxymethyl groups in the C1 position (Fig. 1).

Plants containing pyrrolizidine alkaloids have long been used in folk medicine (Schoental, 1968), but interest in the alkaloids has largely centred on their hepatotoxic activity in livestock (Bull, Culvenor & Dick, 1968) and laboratory investigations of structure-activity relationships have been concerned with this property (Schoental, 1957, 1960; Culvenor, Dann & Dick, 1962; Bull *et al.*, 1968). Some pyrrolizidine alkaloids have spasmolytic and atropine-like properties in isolated intestinal preparations (Chen, Harris & Schulze, 1940a; Chen, Harris & Rose, 1940b; Harris, Anderson & Chen, 1942a, b; Harris, Rose & Chen, 1957; McKenzie, 1958; Rose, Harris & Chen, 1959). Many authors have regarded these properties as being synonymous, however, and the mode of action of the alkaloids is not clearly known.

The principal alkaloid of clinical importance is platyphylline which has been used in Russia as a mydriatic and for the treatment of gastrointestinal hypermotility (Khylstov, 1966). This compound shows typical atropine-like properties such as mydriasis (Chen *et al.*, 1940b; Braslavskaya, 1959), antagonism of pilocarpine and

physostigmine induced miosis (Syrneva, 1946), abolition of vasodepressor responses to acetylcholine and to vagal stimulation (Chen *et al.*, 1940b), inhibition of acetylcholine induced contractions of smooth muscle preparations from the gastrointestinal tract (McKenzie, 1958) and inhibition of increases in salivary secretion produced by pilocarpine (Goldenhershel, 1943 ; Syrneva, 1946).

In the present paper the actions of several pyrrolizidine alkaloids have been tested on the guinea-pig isolated ileum preparation. An attempt has been made to differentiate between the non-specific spasmolytic and the atropine-like actions of

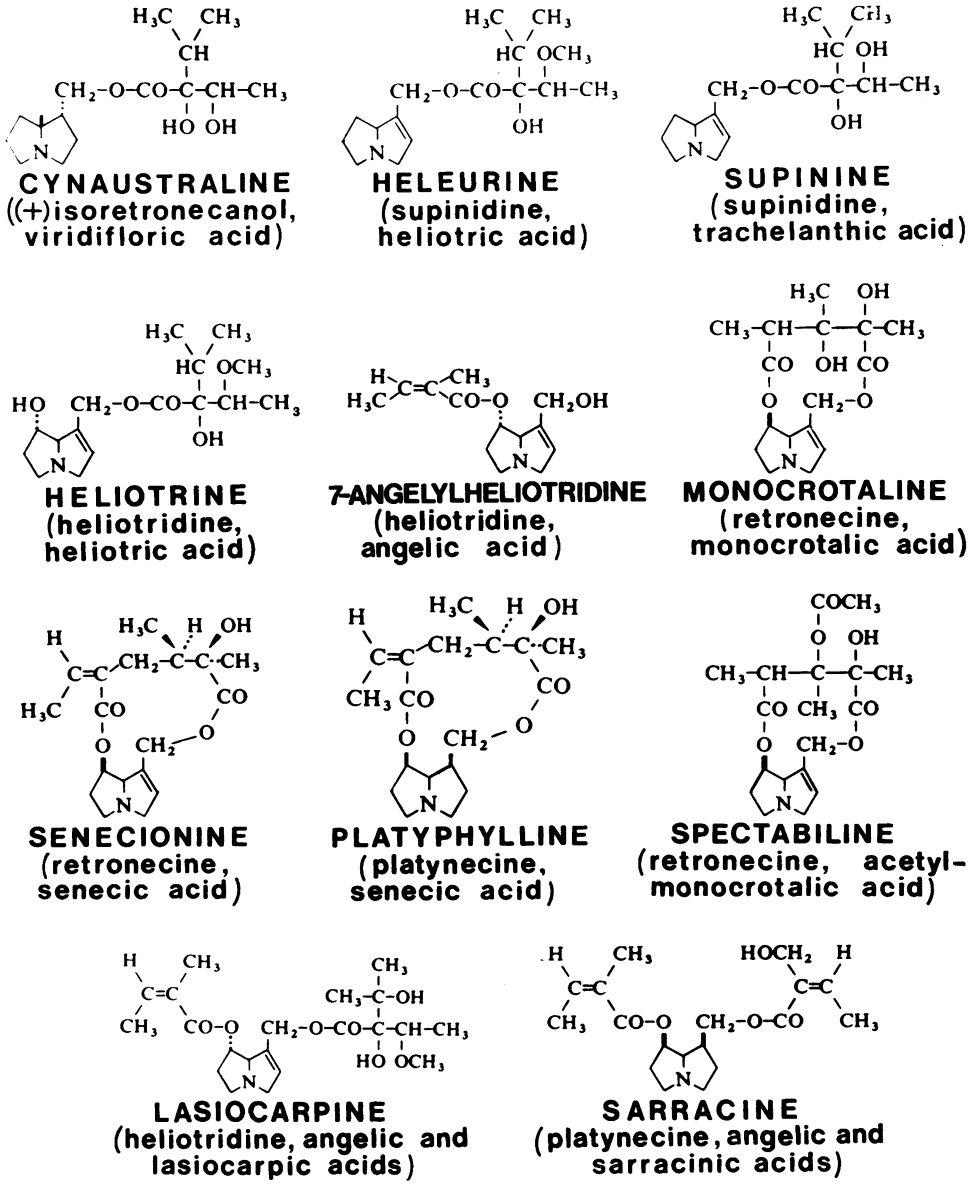

FIG. 1. Structural formulae of the pyrrolizidine alkaloids used in the present study. The amino-alcohol and acid moieties of each ester are shown.

the compounds and to determine how changes in structure affect the latter activity. The compounds were also tested on the toad rectus abdominis preparation.

Methods

Guinea-pigs of either sex were killed by stunning and bleeding. Segments of ileum were suspended in Tyrode solution maintained at 32° C and bubbled with air. Contractions were recorded on a smoked drum with an isotonic frontal writing lever which had a load of between 1 and 2 g and a 10-fold magnification.

Antagonism of histamine and acetylcholine induced responses was evaluated by determining pA_2 values using the method described by Schild (1947). The antagonist was in contact with the tissue for 2 min in these experiments, and quoted pA_2 values are the mean of the results obtained from four to six experiments.

Isolated toad rectus abdominis preparations were suspended in toad Ringers at room temperature and oxygenated with air. The effects of the alkaloids on contractions elicited by acetylcholine (7×10^{-7} to 7×10^{-8}M) were observed.

The following pyrrolizidine alkaloids were used: 7-angelylheliotridine, cynaustraline, heleurine, heliotrine, lasiocarpine, monocrotaline, platyphylline, sarracine, senecionine, spectabiline and supinine. Sarracine and cynaustraline are yellow brown liquids, whereas the remaining alkaloids are white solids. The pure compounds were stored under nitrogen at 0° C. Stock solutions of the alkaloids (10 mg/ml, approximately 5×10^{-2}M) were prepared using a 0·9% w/v sodium chloride solution acidified with hydrochloric acid to give a final solution of pH 2–3. The stock solutions were diluted with Tyrode solution immediately before testing.

The pyrrolizidine alkaloids were supplied by Dr. C. C. J. Culvenor, Division of Applied Chemistry, C.S.I.R.O., Melbourne.

Results

None of the alkaloids had stimulant actions on the guinea-pig ileum preparation in concentrations of up to 2×10^{-3}M. However, all depressed responses to acetylcholine ($3{\cdot}5–7{\cdot}0 \times 10^{-8}$M) and histamine ($1{\cdot}0–2{\cdot}0 \times 10^{-7}$M). The alkaloids fell into two distinct groups: those which abolished responses to acetylcholine in concentrations which had little or no effect on responses produced by histamine, and those which reduced both histamine and acetylcholine responses to a similar extent.

Platyphylline, cynaustraline, supinine and heleurine belong to the first group. Concentrations up to $3{\cdot}5 \times 10^{-6}$M abolished responses to acetylcholine; much higher concentrations ($2{\cdot}6–3{\cdot}5 \times 10^{-4}$M) were needed to affect the responses to histamine. Figure 2a and b shows these effects when platyphylline was used as the antagonist. After wash-out of concentrations of the alkaloids which produced complete abolition of contractions to both histamine and acetylcholine, responses to histamine returned to control levels at a faster rate than those to acetylcholine. The specificity of these compounds for cholinergic receptor sites is indicated by the difference between their pA_2 values against acetylcholine (6·5–7·9) and against histamine (3·8–4·8) (Table 1). Atropine behaved similarly: pA_2 values for acetylcholine and for histamine were 8·6 and 5·7, respectively. Similar pA_2 values for acetylcholine were obtained when these alkaloids were left in contact with the tissue for 5 min instead of only for 2 minutes. It therefore appears that with the above alkaloids equilibrium conditions are attained rapidly.

Senecionine, heliotrine, 7-angelylheliotridine, sarracine, lasiocarpine (Fig. 2c), spectabiline and monocrotaline in concentrations of approximately $2{\cdot}5–4{\cdot}0 \times 10^{-5}$M antagonized responses to acetylcholine and histamine to a similar extent. The duration of the blockade was short lasting after wash-out of the alkaloids and responses to both acetylcholine and histamine returned at a similar rate (Fig. 2c). These alkaloids appear to lack specificity as antagonists (Table 1). These pA_2 values are also within the same range as those found for histamine antagonism with platyphylline, heleurine, supinine and cynaustraline.

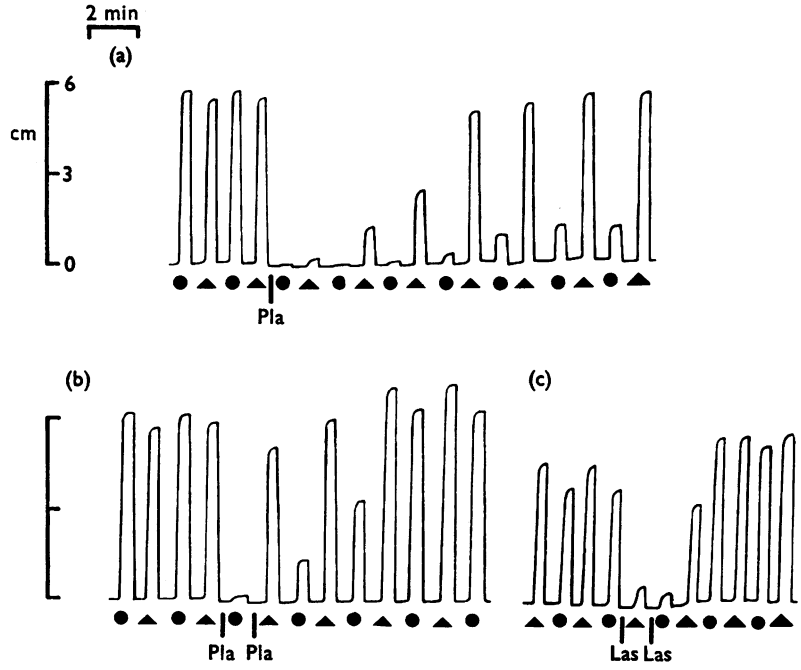

FIG. 2. Responses of guinea-pig ileum preparations to histamine (▲, 15 ng/ml) and acetylcholine (●, 10 ng/ml). Platyphylline (Pla) was used in concentrations of 25 μg/ml (a), and 100 ng/ml (b). In (c) lasiocarpine (Las, 25 μg/ml) was used. Doses of platyphylline and lasiocarpine were given 2 min before the agonists where indicated. Scales for time and contraction height are common in (a), (b) and (c).

TABLE 1. *Antagonistic activities expressed as pA_2 values after the antagonists had been in contact with the tissue for 2 minutes*

Alkaloids	pA_2 ACh	pA_2 Hist.	pKa
Platyphylline	7·9	4·6	9·4
Heleurine	6·8	4·4	9·6
Supinine	6·8	3·8	9·5
Cynaustraline	6·5	4·8	10·6
Senecionine	5·0	4·5	6·8
Heliotrine	4·8	4·1	8·5
7-Angelylheliotridine	4·6	4·4	9·7
Sarracine	4·5	4·5	8·5
Spectabiline	4·5	3·3	7·9
Lasiocarpine	4·5	4·8	7·6
Monocrotaline	3·6	2·8	7·6
Atropine	8·6	5·7	10·0

pKa values were obtained from the work of Bull, Culvenor & Dick (1968), and Culvenor (personal communication).

With the exception of lasiocarpine and sarracine, concentrations of the alkaloids which produced a dose ratio of 2 caused a parallel shift of dose-response curves to acetylcholine without depressing the maximal response. This is consistent with competitive antagonism.

The antagonism produced with platyphylline, supinine, heleurine and cynaustraline was shown to be competitive when carbachol was used as an agonist. Control dose-effect curves to carbachol were obtained, and thereafter points equivalent to approximately 30% and 70% of the control maximal response were produced in the presence of increasing concentrations of the alkaloids. An antagonist contact time of 5 min was used in these experiments.

The dose ratios obtained with various concentrations of the alkaloids are shown in Table 2. The corresponding values of the affinity constant are also shown and the fact that these are fairly constant over a considerable range of dose ratios supports the idea that the antagonism is competitive. The values of log K, with carbachol as agonist, are similar to those of pA_2 with acetylcholine as agonist shown in Table 1.

All the alkaloids in concentrations of up to 3×10^{-4}M were weak or inactive in antagonizing the effects of acetylcholine in the toad rectus abdominis preparation.

Discussion

The present experiments show that the pyrrolizidine alkaloids can be separated into two distinct groups. Platyphylline, cynaustraline, heleurine and supinine have specific and competitive antagonistic activity at muscarinic receptor sites in the guinea-pig ileum. With these alkaloids responses to histamine are depressed at 50–2000 times the concentration required to antagonize responses to acetylcholine.

Senecionine, heliotrine, 7-angelylheliotridine, sarracine, spectabiline, lasiocarpine and monocrotaline only antagonize responses to muscarinic receptor stimulation in concentrations which are between 800 and 20,000 times those of platyphylline which are effective. These concentrations are of the same order as those required to antagonize responses to histamine. This non-specific depressant activity, which requires concentrations of at least $2{\cdot}5\text{–}4{\cdot}0 \times 10^{-5}$M might well be involved in the ability of several of these compounds to depress spontaneous pendular movements

TABLE 2. *Mean dose ratios and affinity constants (K) obtained with various concentrations of the pyrrolizidine alkaloids*

	Molar concentration	Dose ratio	K
Platyphylline	$1{\cdot}5 \times 10^{-7}$	11·7	$7{\cdot}13 \times 10^{7}$
	$1{\cdot}5 \times 10^{-6}$	83·0	5·47
	$1{\cdot}5 \times 10^{-5}$	478·0	3·18
	$7{\cdot}5 \times 10^{-5}$	2740·0	3·65
Supinine	$1{\cdot}75 \times 10^{-6}$	7·7	$3{\cdot}83 \times 10^{6}$
	$3{\cdot}5 \times 10^{-6}$	15·0	4·00
	$1{\cdot}75 \times 10^{-5}$	44·7	2·50
	$8{\cdot}75 \times 10^{-5}$	173·0	1·96
Heleurine	$1{\cdot}75 \times 10^{-6}$	6·2	$2{\cdot}97 \times 10^{6}$
	$1{\cdot}75 \times 10^{-5}$	37·3	2·07
	$8{\cdot}75 \times 10^{-5}$	174·0	1·97
Cynaustraline	$3{\cdot}5 \times 10^{-6}$	6·6	$1{\cdot}6 \times 10^{6}$
	$3{\cdot}5 \times 10^{-5}$	37·7	1·05
	$8{\cdot}75 \times 10^{-5}$	81·0	0·91
	$1{\cdot}75 \times 10^{-4}$	169·0	0·96

Each alkaloid was tested at the indicated concentrations in a single preparation. Dose ratios were obtained and the quoted mean results were calculated from four such experiments with each alkaloid.

in the isolated rabbit and rat intestine (McKenzie, 1958 ; Harris *et al.*, 1942a, b ; Rose *et al.*, 1959).

In Fig. 3, the probable conformation of atropine and its binding to the muscarinic receptor site is shown. The drug-receptor interaction involves binding of the nitrogen at the anionic receptor site (A) and the ether and carbonyl oxygens at the esteratic sites (B and C). In addition, the tropyl hydroxy group of atropine may bind to a further site (E) located close to the site responsible for binding of the carbonyl oxygen. The benzene ring of the tropic acid moiety occupies the critical accessory receptor area (D). These receptor binding areas are based on the work of various authors who have studied the effects of agonists (Barlow, 1964 ; Bebbington & Brimblecombe, 1965 ; Beckett, 1967) and antagonists (Long, Luduena, Tullar & Lands, 1956 ; Barlow, 1964 ; Ellenbrock, Nivaard, van Rossum & Ariens, 1965 ; Ariens & Simonis, 1967) at muscarinic receptor sites.

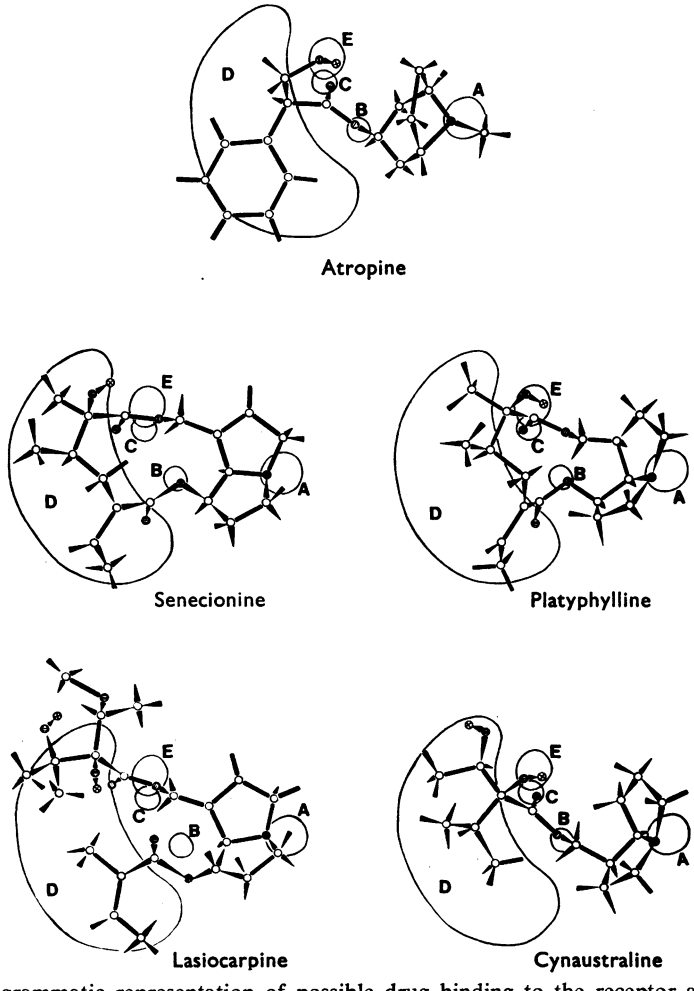

FIG. 3. Diagrammatic representation of possible drug binding to the receptor areas involved in anticholinergic activity at muscarinic receptor sites. The conformational diagrams were adapted from photographs taken vertically above molecular models based on known configurations of the pyrrolizidine alkaloids. (○), Carbon atoms ; (●), nitrogen atoms ; (◉), oxygen atoms ; (×), hydrogen atoms of hydroxyl groups. Other hydrogens on carbon atoms are not shown. A, B, C, D and E show possible binding areas as specified in the text.

As with atropine, the pyrrolizidine alkaloids used in the present experiments contain a tertiary nitrogen atom, ester linkages, hydroxyl and alkyl groups. These moieties can be regarded as essential molecular features in potent antagonists at muscarinic receptor sites.

At physiological pH, the nitrogen within the pyrrolizidine nucleus exists in a charged form. A comparison of the anti-cholinergic potencies and the pKa values of the pyrrolizidine alkaloids (Table 1) suggests that there is no direct correlation between the concentration of the charged form of the alkaloids and their antagonistic activity.

McKenzie (1958) has suggested that ring saturation of the pyrrolizidine nucleus is an important requirement for anticholinergic activity. However, the present experiments indicate that the degree of saturation has variable effects on the atropine-like properties of the alkaloids. Thus, saturation of the pyrrolizidine ring structure in senecionine to produce platyphylline leads to an 800-fold increase in anticholinergic activity and it also leads to a big increase in ionization (pKa increases from 6·8 to 9·4). The presence or absence of ring saturation in the compounds supinine, cynaustraline and heleurine, however, causes little change in the potency of the compounds as atropine-like drugs.

Although ring saturation has variable effects on the atropinic actions, the presence of an unsaturated pyrrolizidine nucleus appears to be an essential prerequisite for a hepatotoxic action of the alkaloids. A comparison of data from toxicity studies (Bull *et al.*, 1968 ; Schoental, 1968) with the present results suggests that there is no correlation between hepatotoxic and anticholinergic activity.

Substitutions on the pyrrolizidine nucleus profoundly affect ability to antagonize acetylcholine. Heleurine, cynaustraline and supinine all have specific anticholinergic activity and have a single substituent group on the C1 position of the pyrrolizidine ring. Free rotation within the acidic moiety of the molecule is possible in these alkaloids because of the absence of substituents on C7 of the pyrrolizidine nucleus.

Configurations of the alkaloids have been deduced from analysis of chemical synthetic and degradation products, nuclear magnetic resonance spectra and X-ray crystallography (Bull *et al.*, 1968 ; Culvenor, personal communication). Molecular models of the alkaloids based on their known configurations show that heleurine, supinine and cynaustraline may adopt a conformation which allows attachment to all the binding sites proposed for muscarinic receptor antagonists. Figure 3 shows the possible drug-receptor interaction of cynaustraline at this receptor.

In general, further substitution on the pyrrolizidine ring leads to a loss of ability to antagonize acetylcholine. Senecionine, monocrotaline, spectabiline and platyphylline all possess substituents which form a macrocyclic ring structure bridging the C1–C7 position of the pyrrolizidine nucleus. Platyphylline is the only compound of this type to have specific atropine-like activity.

The conformation of platyphylline (Fig. 3) allows close apposition to binding areas of the muscarinic receptor site and to the proposed accessory areas critical for anticholinergic activity. Structurally, senecionine, which lacks specific antagonistic activity, differs from platyphylline only in the presence of a double bond within the amino-alcohol moiety. This double bond induces a more planar configuration in the molecule, reducing flexibility and diminishing the probability of binding of the carbonyl group at site C and the hydroxyl group at site E (Fig. 3).

Lasiocarpine (Fig. 3), heliotrine, 7-angelylheliotridine and sarracine have substituents on both the C1 and C7 positions of the pyrrolizidine nucleus. These compounds do not antagonize acetylcholine and this may be related to steric hindrance between the groupings attached at position C1 and C7, which may reduce attachment of the molecules to acetylcholine receptors.

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