Phytochemistry, Vol 28, No 10, pp 2631–2634, 1989
Printed in Great Britain

0031–9422/89 $3.00 + 0.00
Pergamon Press plc

THE ACETYLCHOLINESTERASE-INHIBITORY ACTIVITY OF STEROIDAL GLYCOALKALOIDS AND THEIR AGLYCONES

JAMES G. RODDICK

Department of Biological Sciences, University of Exeter, Exeter, U K

(*Received* 23 *January* 1989)

Key Word Index—*Solanum* sp; Solanaceae, acetylcholinesterase inhibition, glycoalkaloids, α-solanine, α-chaconine, β_2-chaconine, tomatine, solasonine, solamargine, aglycones; solanidine, tomatidine, solasodine

Abstract—The steroidal glycoalkaloids α-solanine and α-chaconine significantly inhibited bovine and human acetylcholinesterase at a concentration of 100 μM, but their effects were not pH dependent β_2-Chaconine was as effective as α-chaconine. Tomatine was less inhibitory, whereas solasonine and solamargine showed very much reduced activity Longer incubation times did not intensify the enzyme inhibition The aglycones solanidine, tomatidine and solasodine produced only slight to negligible inhibition which did not change with incubation time Combinations of solanine and chaconine and also of solasonine and solamargine produced effects which were slightly antagonistic or non-interactive. None of the glycoalkaloids approached the non-steroidal alkaloid physostigmine in inhibitory action The non-nitrogenous steroidal saponin digitonin and the non-steroidal saponins of *Gypsophila*, *Quillaja* and *Glycyrrhiza* were not inhibitory to these enzymes

INTRODUCTION

Steroidal glycoalkaloids are a group of physiologically active, saponin-like secondary compounds prominent in species of *Solanum* [1]. The glycoalkaloids of potato (*S. tuberosum*) have been responsible for numerous instances of human and livestock poisonings and fatalities [2] and are also known to impair feeding activity and development in various insect species [3] as well as growth of certain fungi [4] Consequently, these compounds are of both medical and ecological significance. In animals, adverse physiological effects of steroidal glycoalkaloids are manifest in a number of ways (e.g reduced respiratory activity or blood pressure, bradykardia, haemolysis, etc) which are thought to stem mainly from membrane disruption, inhibition of acetylcholinesterase or interference with sterol/steroid metabolism, or from combinations of these.

In many *Solanum* species, two major, chemically related glycoalkaloids are elaborated, but most studies on biological activity have been concerned with the effects of individual compounds. In recent investigations of the membrane-lytic properties of the potato glycoalkaloids α-solanine and α-chaconine using both synthetic membrane vesicles [5] and cells of animal, plant and fungal origin [6], we demonstrated not only that chaconine was the more active alkaloid, but that, in combination, the two compounds gave rise to a synergistic lytic effect. In the context of chemical defence, synergistic interactions may have important implications for the stability and metabolic cost of the system

The anti-cholinesterase activity of solanaceous glycoalkaloids was first demonstrated by Pokrovskii [7] and has since been confirmed by a number of workers both *in vitro* and *in vivo* [e g 8–12]. However, no information appears to exist on whether naturally paired glycoalkaloids interact in their effects on acetylcholinesterase activity, as they do on membrane integrity. Experiments were therefore conducted to examine this possibility using solanine/chaconine and also solasonine/solamargine In addition, the effects of other glycoalkaloids, various aglycones, some non-nitrogenous saponins and a non-steroidal alkaloid were studied with a view to gaining further information on the relative effectiveness of steroidal glycoalkaloids against acetylcholinesterase and on the contribution of their aglycone and carbohydrate moieties.

RESULTS

In view of the well-documented high-pH-enhancement of glycoalkaloid disruption of membranes [e.g. 13, 14], this parameter was included in initial experiments to assess the dose-response relationship of potato glycoalkaloids to bovine cholinesterase. Controls suggested that cholinesterase activity was reduced at pH 5 (see Fig. 1 legend), although pH appeared not to exert a major influence on cholinesterase inhibition by solanine and chaconine (Fig. 1). Negative values for inhibition at low alkaloid concentrations resulted from values representing cholinesterase activity (ΔA_{412}/min) being slightly (but not significantly) greater than those of controls Both glycoalkaloids were inhibitory to approximately the same degree, with a minimum effective concentration between 1 and 10 μM Inhibition by solanine and chaconine was of the order of 30–35% at 10 μM, rising to *ca* 75% at 100 μM (Fig. 1)

A comparison of the effects of solanine and chaconine on bovine and human cholinesterase (at standard pH and concentration) with the glycoalkaloids β_2-chaconine, solasonine, solamargine and tomatine and the non-steroidal alkaloid physostigmine (eserine), yielded data shown in Fig. 2. Physostigmine was by far the most inhibitory compound tested, causing 40–73% inhibition at 1 μM and 100% inhibition at 10 μM. Solanine, chaconine and

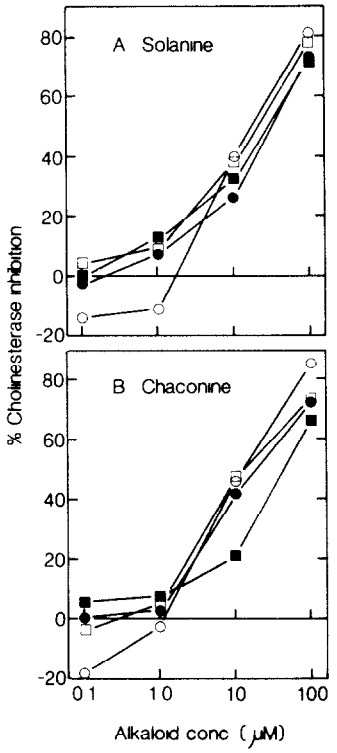

Fig 1 Semi-log plot showing the effect of concentration of (A) solanine and (B) chaconine on inhibition of bovine acetylcholinesterase activity at different pH pH was varied by using different 0·1M phosphate buffers (pH 6, 7 and 8) and 0·1M phosphate–citrate buffer (pH 5) Values for enzyme activity (ΔA_{412}/min) in blanks lacking glycoalkaloid at pH 5, 6, 7 and 8 were 0·042, 0·20, 0·15 and 0·23 respectively ○, ●, □ and ■ represent pH 5, 6, 7 and 8 respectively Each point is the mean of five replicates The s e range was ± 1·48–8·93

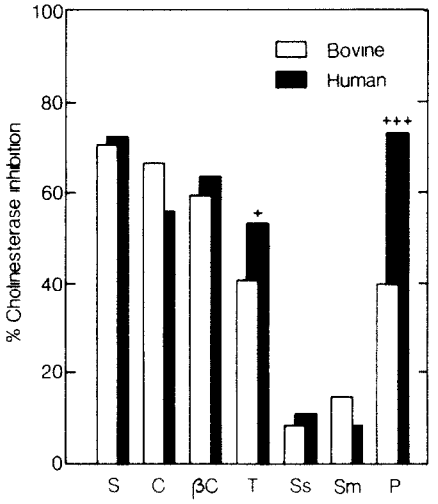

Fig 2 Comparison of the effects of different alkaloids on inhibition of bovine (open columns) and human (closed columns) acetylcholinesterase at pH 7 S = solanine, C = chaconine, βC = β_2-chaconine, T = tomatine, Ss = solasonine, Sm = solamargine, P = physostigmine All treatments were 100 μM at pH 7 with the exception of physostigmine which was 1 μM Values represent the mean of five replicates The s e range was ± 0·67–5·52 +, + + + = significantly different from effect on bovine enzyme at $p = 0·05$ and 0·001 respectively

β_2-chaconine were less inhibitory (by more than a factor of 10), but all showed much the same inhibitory activity against both enzymes Tomatine had significant anti-cholinesterase activity, although it was consistently less than that of the potato glycoalkaloids There was also some indication of a greater effect of tomatine and physostigmine on human cholinesterase than on bovine cholinesterase. Solasonine and solamargine, on the other hand, showed very much reduced activity against both enzymes Treatment of bovine and human cholinesterase with solanine, chaconine and tomatine for varying periods of time up to 2 hr did not result in any intensification of inhibition (data not presented)

A comparison was made of the individual and combined effects of solanine and chaconine, and of solasonine and solamargine, on bovine cholinesterase using maximum concentrations of 10 and 100 μM respectively A lower concentration was used with the more active solanine and chaconine to avoid masking any synergism between these compounds in combination. The data in Table 1 relating to inhibition by individual compounds are generally comparable with those in Figs 1 and 2 The values for the combined treatments provide no evidence of any synergism in inhibitory action but, if anything, slightly antagonistic or non-interactive effects

Table 1 Effects of solanine/chaconine and solasonine/solamargine individually and in combination on inhibition of bovine cholinesterase at pH 7

Treatment	Inhibition (%)
Solanine (10 μM)	44·3
Chaconine (10 μM)	54·4
Solanine (5 μM)	27·7
Chaconine (5 μM)	25·9
Solanine (5 μM) + chaconine (5 μM)	37·3
Solasonine (100 μM)	16·7
Solamargine (100 μM)	13·0
Solasonine (50 μM)	−12·0
Solamargine (50 μM)	−3·6
Solasonine (50 μM) + solamargine (50 μM)	0·5

The different concentrations used reflect the different inhibitory activity of the two groups of glycoalkaloids (see Fig. 2 and text) Each value is the mean of the five replicates The s e range was ± 1·63–9·95

A study of the effects of solanidine, tomatidine and solasodine (the aglycones of solanine/chaconine, tomatine and solasonine/solamargine respectively) on bovine and human cholinesterase yielded rather variable (including negative) values (Table 2) The latter resulted from low to negligible inhibitory effects coupled with natural variability, as indicated above Overall, there was no evidence of significant inhibition by these compounds either in the standard assay (0 hr incubation) or following longer

Table 2 Effects of solanidine, tomatidine and solasodine (all 100 μM) on inhibition of bovine and human cholinesterase activity at pH 7 following various incubation periods

Source of cholinesterase	Incubation time (hr)	% Inhibition by		
		Solanidine	Tomatidine	Solasodine
Bovine	0	11 1	15 6	8 9
	1	5.7	−7 7	−15 0
	2	2 0	−4 5	−1.5
Human	0	−6 2	−13 3	−8 3
	1	3.6	−13 8	−2 4
	2	14 1	−10 6	0 6

In all treatments, 3 min was allowed between addition of the last component and measurement of absorbance. In the 1 hr and 2 hr treatments, acetylthiocholine iodide and dithiobis-2-nitrobenzoic acid were added at the end of the incubation period Data are means of five replicates Negative values result from activity in treatments being slightly (but not significantly) greater than in controls The s e. range was ± 0.91–9.90.

incubation times (Table 2) Similar experiments to examine the effects of non-nitrogenous saponins such as digitonin, glycyrrhizin and *Gypsophila* and *Quillaja* saponins revealed no inhibitory or promotory effects of these compounds on either bovine or human cholinesterase with incubation times of up to 2 hr (data not presented)

DISCUSSION

The inhibitory effects of solanine and chaconine on bovine and human cholinesterase showed three major differences from their lytic action on membranes, viz. (i) pH was not a major determining factor, (ii) both glycoalkaloids were equally active and (iii) no synergism occurred between the two compounds The lack of a pH effect is not entirely surprising since in membrane lysis it apparently derives from the pH-dependent sterol-binding properties of glycoalkaloids [15]. The lack both of differential effects of solanine and chaconine on cholinesterase and of a synergism between them may be seen as related events if the evolution of a synergism (in relation to membrane disruption) compensates for the reduced activity of solanine. Consistent with these findings is that solasonine and solamargine, which also show evidence of a synergism in their effects on membranes (paper in preparation), did not so interact with regards to inhibition of cholinesterase.

The data presented here reveal some interesting comparisons with earlier work on glycoalkaloid inhibition of cholinesterase activity For example, Harris and Whittaker [9] reported inhibition of serum cholinesterase of up to *ca* 80% by 3 μM solanine and solanidine, while Orgell [10] recorded 50% inhibition of the same enzyme by 5 μM solanine. None of the effects in this communication were anything like so potent and the maximum inhibition observed (*ca* 85%) required *ca* 100 μM glycoalkaloid. The reasons for these discrepancies are not clear, but the greater purity of the compounds used here (e g Sigma solanine and chaconine are *ca* 98% pure [16]) could be important, as could the source and sensitivity of the enzymes Harris and Whittaker [17] identified three distinct types of human cholinesterase based on susceptibility to steroidal alkaloids. The levels of inhibition reported here are more of the order of those recently found by Bushway *et al.* [12] (who also used highly-purified alkaloids), although somewhat higher, with 40 μM solanine and chaconine producing about 55% inhibition of bovine cholinesterase compared with *ca* 26% inhibition of eel cholinesterase [12]. Bushway *et al.* [12] found negligible inhibition of this enzyme by tomatine, although it was tested only at 33 μM. A comparable concentration was not used here, but data from a 100 μM tomatine treatment indicate that this glycoalkaloid is capable of effectively inhibiting mammalian cholinesterase. A quantitatively similar finding was reported by Orgell [10].

The much reduced inhibitory activity of the solasodine-based glycoalkaloids solasonine and solamargine which share the same trioside carbohydrate moieties as the solanidine-based solanine and chaconine respectively confirms the importance of the nature of the aglycone moiety in anticholinesterase activity. Although it might appear from the finding that β_2-chaconine (which lacks one rhamnose) is as inhibitory as α-chaconine that the carbohydrate moiety is less important, the minimum to negligible activity of solanidine and tomatidine suggests that the carbohydrate component does make a significant contribution to anticholinesterase activity. These conclusions are generally in keeping with those of Bushway *et al.* [12] based on comparisons of α-and β_2-chaconine and tomatine, demissine and commersonine. The inability of various non-nitrogenous saponins (including the steroidal saponin, digitonin) to depress cholinesterase activity further indicates that inhibition of this enzyme does not derive simply from surface activity or from possession of a steroid structure. It would seem that the heterocyclic nitrogen of steroidal alkaloids is an important feature [11].

The *in vitro* anticholinesterase activity of certain steroidal glycoalkaloids, although much lower than alkaloids such as physostigmine [see also 10, 11], could still contribute to the toxic effects exerted by these compounds on mammals and insects *in vivo* [11], especially as local concentrations in plant cells may be up to × 10 greater than the maximum tested here [18].

EXPERIMENTAL

Test alkaloids and saponins α-Solanine, α-chaconine, tomatine, solanidine, tomatidine, solasodine, physostigmine and digitonin were purchased from Sigma, Poole, U K Solasonine/solamargine, β_2-chaconine, and *Gypsophila* saponins, *Quillaja* saponins and glycyrrhizin were gifts from Dr M Weissenberg (ARO, Bet Dagan, Israel), Dr S F Osman (USDA, Philadelphia, U.S A) and Drs G R Fenwick and K R Price (AFRC Institute of Food Research, Norwich, U K) respectively

Estimation of acetylcholinesterase inhibition The spectrophotometric method used was based on that of Ellman *et al* [19] To a 1 cm path length silica cuvette were added, in order, 2 8 ml 0 1 M Pi buffer, pH 7 0, 200 μl test compound prepared as indicated below, 20 μl 0 075M acetylthiocholine iodide (Sigma), 100 μl 0 1 M Pi buffer, pH 7 0, containing 0 01M 5,5-dithiobis-2-nitrobenzoic acid (Sigma) and 1 5 mg/ml $NaHCO_3$, and finally 50 μl aq soln (5 units/ml) of acetylcholinesterase from bovine erythrocytes (Sigma type XII-S) or human erythrocytes (Sigma, type XIII) Controls contained all components except the test compounds Contents were mixed, left for exactly 3 min at room temp. and A_{412} measured on a recording spectrophotometer for at least 2 min Enzyme activity was expressed as ΔA_{412}/min All treatments consisted of 5 replicates and each experiment was carried out at least twice Where appropriate, statistical significance was tested using a *t*-test

Preparation of solutions of test compounds Stock solns of α-solanine, α-chaconine, β_2-chaconine, tomatine, solasonine, solamargine, physostigmine, glycyrrhizin and *Gypsophila* and *Quillaja* saponins were prepared by dissolving in 0 04 ml 0 1 M HCL and making to 2 ml with dist H_2O Solanidine, tomatidine, solasodine and digitonin were dispersed in DMSO which was diluted to 16% with dist H_2O The final DMSO concentration in the reaction mixture was 1% and this had no effect on cholinesterase activity

Acknowledgements—The author is indebted to Drs G R Fenwick, S F Osman, K R Price and M Weissenberg for gifts of glycoalkaloids and saponins as indicated in the Experimental section, and to Anna Rijnenberg for excellent technical assistance

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