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ANTAGONIST PROPERTIES OF ARYLAMINOPYRIDAZINE GABA DERIVATIVES AT THE *ASCARIS* MUSCLE GABA RECEPTOR

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Summary

1. In a previous study, it was shown that the potency order for two arylaminopyridazine derivatives, SR95531 and SR95103, was different in *Ascaris suum* when compared to vertebrate preparations. SR95531, the most potent analogue at the vertebrate $GABA_A$ receptor, was found to be very weak at antagonizing GABA responses in *Ascaris*, but SR95103, approximately 20 times less potent than SR95531 in vertebrate preparations, was more potent than SR95531 in *Ascaris*. These results suggested the existence of different accessory binding sites at the *Ascaris* GABA receptor.

2. To test this hypothesis, the effects of a series of arylaminopyridazine derivatives of GABA on the GABA response in *Ascaris suum* muscle were investigated using a two-microelectrode current-clamp technique.

3. The results showed that SR42627, a potent antagonist at the $GABA_A$ receptor, was one of the weakest analogues in *Ascaris* muscle. In contrast, SR95132, virtually inactive in vertebrate preparations, was equipotent to SR95103, the most potent analogue of the series in *Ascaris* muscle.

4. The three most potent analogues in *Ascaris*, SR95103, SR95132 and SR42666, displace GABA dose–response curves to the right without decreasing the maximal response. The modified Schild plots for these compounds are consistent with a competitive mechanism involving two molecules of GABA and only one molecule of antagonist interacting with the receptor. The estimated dissociation constants for SR95103, SR95132 and SR42666 are, respectively, 64, 65 and 105 $\mu mol\,l^{-1}$.

5. Structure–activity relationships for this series of compounds were examined in *Ascaris* and compared to those in vertebrates. Substitution on the pyridazine ring in the 4-position, while detrimental for the antagonist potency at the vertebrate $GABA_A$ receptor, appears to be a prerequisite for antagonistic activity on the *Ascaris* muscle GABA receptor. These results are interpreted in terms of the accessory binding site theory of Ariëns, and suggest the existence of different accessory binding sites on the *Ascaris* GABA receptor.

Key words: *Ascaris suum*, GABA, arylaminopyridazine GABA derivatives, antagonist.

Introduction

The GABA receptor has been described as a target for pesticide drugs (Eldefrawi and Eldefrawi, 1987) and anthelmintic drugs (Martin, 1982; Martin and Pennington, 1989). In the nematode parasite *Ascaris suum*, two well-known anthelmintics have been shown to interact with GABA receptors: piperazine (Del Castillo *et al.* 1963, 1964*a*; Martin, 1982, 1985) and ivermectin (Holden-Dye *et al.* 1988; Martin and Pennington, 1989). Pharmacological characterization of the *Ascaris* GABA receptor shows that, in terms of agonist properties, it is closely related to vertebrate $GABA_A$ receptors (Holden-Dye *et al.* 1989). This contrasts with the antagonist properties, since none of the classical $GABA_A$ antagonists are potent on *Ascaris*. Bicuculline, picrotoxin, securinine, pitrazepine, TBPS (tert-butylbicyclophosphothionate) and dieldrin are either weak or inactive GABA antagonists in *Ascaris* (Holden-Dye *et al.* 1988; Colqhoun *et al.* 1989). In agreement with these results, the arylaminopyridazine GABA derivative and potent $GABA_A$ antagonist SR95531 was shown to be very weak at antagonizing GABA responses in *Ascaris* (Holden-Dye *et al.* 1989; Duittoz and Martin, 1991*a*). However, its close analogue SR95103 was shown to antagonize GABA responses in a competitive manner with an apparent dissociation constant (K_B) of 64 $\mu mol\,l^{-1}$ (Duittoz and Martin, 1991*a*,*b*). This contrasts with the vertebrate $GABA_A$ receptor, at which SR95531 is the most potent antagonist, being approximately 20 times more potent than SR95103 (Heaulme *et al.* 1986*b*). It was then suggested that arylaminopyridazine GABA derivatives would show structure–activity relationships in *Ascaris* different from those found at the vertebrate $GABA_A$ receptor (Duittoz and Martin, 1991*a*). In this study, other arylaminopyridazine derivatives were tested (Fig. 1); their relative potency in *Ascaris* muscle was compared to that in mammalian preparations, and the results are discussed in terms of accessory binding site theory (Ariëns *et al.* 1979).

Materials and methods

Ascaris suum were collected from the local abattoir and kept in Locke's solution at 37°C. They were used within 4 days. The muscle flap preparation, made as described by Martin (1980), was pinned down in an experimental chamber maintained at 22°C, and perfused with a high-chloride and low-calcium Ringer's solution containing (in $mmol\,l^{-1}$): NaCl, 135; KCl, 3; $MgCl_2$, 15.7; glucose, 3; Tris, 5; pH adjusted to 7.6 with maleic acid.

Two glass microelectrodes (10–30 MΩ), filled with 2 $mol\,l^{-1}$ potassium acetate, were introduced into the bag region of a muscle cell; one was used to inject hyperpolarizing current pulses (40 nA, 2 s, 0.2 Hz), the other to record the membrane potential. The signals were recorded and amplified with an Axoclamp 2A, monitored on a Tektronix 2210 oscilloscope and played back on a Lectromed chart recorder. The input conductance of the bag was calculated from the voltage response to the injected current pulse; the I/V relationship is sufficiently linear during the injection of hyperpolarizing current to allow this (Martin, 1980).

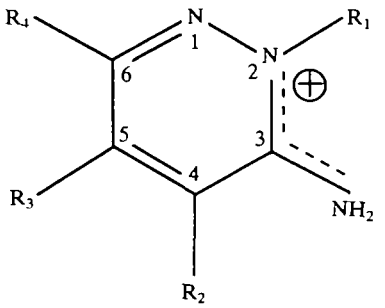

Fig. 1. Structure of arylaminopyridazine GABA derivatives.

	Substituents			
Name	R1	R2	R3	R4
SR95103	$-C_3H_6COOH$	$-CH_3$	-H	$-C_6H_5$
SR95531	$-C_3H_6COOH$	-H	-H	$-C_6H_4OCH_3$
SR42666	$-C_3H_6COOH$	$-CH_3$	-H	$-C_6H_4OCH_3$
SR95132	$-C_3H_6COOH$	$-C_6H_5$	-H	$-C_6H_5$
SR42640	$-C_3H_6COOH$	-H	$-CH_3$	$-C_6H_5$
SR42627	$-C_3H_6COOH$	$-CH_3$	-H	$-C_6H_4CH_3$
SR95133	$-C_3H_6COOH$	$-CH_3$	-H	$-C_{10}H_8$

The preparation was not perfused continuously because of the limited quantities of antagonist available. To detect any antagonist activity, 30 μmol l^{-1} GABA was applied first and, when the membrane conductance stabilized to its new value, the bath solution was replaced by 30 μmol l^{-1} GABA+1 mmol l^{-1} antagonist. Antagonists found to be reasonably potent using this method were then tested at several concentrations on GABA dose–response curves. GABA dose–response curves were obtained by cumulative application of increasing GABA concentrations, without intermediate washing. The method involved gentle draining of the bath (2.5 ml) by suction, and then flushing the bath with 5 ml of the next GABA concentration. The antagonist was applied simultaneously with the first GABA concentration, and remained present at subsequent GABA concentrations. A control GABA dose–response curve without antagonist was always obtained from the same cell and dose ratios were calculated (see below).

Dose–response relationships were described by the modified Hill equation (Nistri and Constanti, 1979):

$$\Delta G = \frac{\Delta G_{max}}{1 + (EC_{50}/[X_A])^n},$$

where ΔG is the change in membrane input conductance, ΔG_{max} is the maximal response, EC_{50} is the GABA concentration producing 50% of the maximal response, $[X_A]$ is the GABA concentration and n is the Hill coefficient. A nonlinear regression program (patternsearch, Colquhoun, 1971) was used to

estimate, ΔG_{max}, EC_{50} and n. The action of 1 mmol l^{-1} antagonist on the change in membrane input conductance induced by 30 μmol l^{-1} GABA was examined. The percentage of antagonism was then calculated:

$$\text{percentage of antagonism} = 100[1-(\Delta G_{ant}/\Delta G_c)],$$

where ΔG_c is the change in membrane input conductance produced by 30 μmol l^{-1} GABA and ΔG_{ant} is the change in membrane input conductance produced by 30 μmol l^{-1} + 1 mmol l^{-1} antagonist, measured after a 10 min application of the antagonist. The most potent compounds were then tested at concentrations of 100, 300 and 1000 μmol l^{-1} on GABA dose–response curves. Dose–response curves were considered parallel if the Hill coefficient and ΔG_{max} for the control GABA dose–response curve and the dose–response curves in the presence of the antagonist were not significantly different. Dose ratios were then determined from the ratios of the EC_{50} values measured and examined using the Schild analysis (Arunlakshana and Schild, 1959):

$$\log(DR-1) = \log[X_B] + pK_B,$$

where DR is the dose ratio, $[X_B]$ is the antagonist concentration and pK_B is the negative logarithm of the dissociation constant of the antagonist–receptor complex. However, the classical Schild plot does not account for a stoichiometry greater than 1 between GABA and its receptor. Previous studies on the *Ascaris* muscle GABA receptor (Holden-Dye *et al.* 1988, 1989), and more generally on invertebrate GABA receptors and vertebrate $GABA_A$ receptors (see Nistri and Constanti, 1979, for a review; Schofield, 1989), have suggested that two molecules of GABA are likely to interact with the receptor. To account for positive cooperativity between GABA and its receptor we used a modified Schild analysis (Williams *et al.* 1988):

$$\log(DR^n-1) = M\log[X_B] + pK_B,$$

where n is the Hill coefficient, M is the number of antagonist molecules interacting with the receptor, and the other symbols are explained above.

Means and standard errors (S.E.) were calculated. Statistical significance was assessed using a two-tailed independent t-test.

GABA was obtained from Sigma. SR95103, 2-(3-carboxypropyl)-3-amino-4-methyl-6-phenyl pyridazinium chloride; SR95531, 2-(3-carboxypropyl)-3-amino-6-*p*-methoxy phenyl pyridazinium chloride; SR42666, 2-(3-carboxypropyl)-3-amino-4-methyl-6-*p*-methoxy phenyl pyridazinium chloride; SR95133, 2-(3-carboxypropyl)-3-amino-4-methyl-6-α-napthyl pyridazinium chloride; SR95132, 2-(3-carboxypropyl)-3-amino-4-phenyl-6-phenyl pyridazinium chloride; SR42627, 2-(3-carboxypropyl)-3-amino-4-methyl-6-*p*-methyl phenyl pyridazinium chloride; and SR42640, 2-(3-carboxypropyl)-3-amino-5-methyl-6-phenyl pyridazinium chloride were gifts from Sanofi Recherche.

Results

The results reported here are based on the analysis of experiments recorde

from 32 cells from 19 preparations. Cells selected for recording and analysis had a resting membrane potential greater than −20 mV and a resting input conductance between 1.9 and 4.0 μS. Recordings were rejected if the resting input conductance failed to return to at least 80 % of the control value after the final wash.

Arylaminopyridazine derivatives do not affect the membrane conductance directly. SR95103, SR95531, SR42666, SR42640, SR42627, SR95133 and SR95132 were initially applied at 1 mmol l^{-1} in the absence of GABA. No change in membrane conductance was produced. The lack of activity of arylaminopyridazine derivatives on membrane conductance showed that they do not have agonist properties on GABA receptors, in spite of the GABA side chain in their structure.

Fig. 2 illustrates the effects produced by 1 mmol l^{-1} SR42640, SR42627, SR95133, SR42666 and SR95132 on the response to 30 μmol l^{-1} GABA; Table 1 summarizes the percentage of antagonism. SR42640 had the weakest effect and only antagonized 15 % of the response in Fig. 2A. Following the application of SR42640, the membrane hyperpolarized slightly (Fig. 2A); this is believed to be an artefact due to the application of the drug. SR42627 was not very potent, with only 29 % of the GABA response being antagonized in Fig. 2B. SR95133, the α-napthyl derivative, was more potent than SR95531 (Duittoz and Martin, 1991*a*). The potent antagonists were SR42666, the 4-phenyl derivative SR95132 and SR95103.

All these results are summarized and compared to results obtained in vertebrate preparations in Table 1. The results obtained in *Ascaris* and with vertebrates are difficult to compare quantitatively since they involve different techniques: an

Table 1. *Comparison of the potencies of some arylaminopyridazine GABA derivatives in vertebrate and in* Ascaris suum *muscle*

GABA derivative	Vertebrate K_B (μmol l^{-1})*	*Ascaris* muscle	
		Antagonism (%)†	K_B (μmol l^{-1})‡
SR95531	0.15	44.2±6.1 (N=5)	–
SR42666	0.31	76.6±6.6 (N=5)	104.8±19.7 (N=13)
SR42627	0.5	29, 13 (N=2)	–
SR95103	2.20	92.8±3.2 (N=5)	63.8±12.9 (N=14)
SR95133	2.60	58.0±8.0 (N=4)	–
SR42640	10.40	15, 5 (N=2)	–
SR95132	100.00	89.0±3.4 (N=5)	65.0±20.3 (N=9)

Values are mean±S.E.

*Measured by [^{3}H]GABA displacement from rat brain membranes; K_B, dissociation constant (Wermuth *et al.* 1987).

†Percentage antagonism of the response to 30 μmol l^{-1} GABA, produced by 1 mmol l^{-1} antagonist, in *Ascaris* muscle.

‡ K_B, dissociation constant, estimated from the modified Schild analysis, in *Ascaris*. It was not possible to measure quantitatively the antagonism of the low-potency analogues (SR42640, SR42627, SR95133 and SR95531) because the amount required exceeded that available for experimentation.

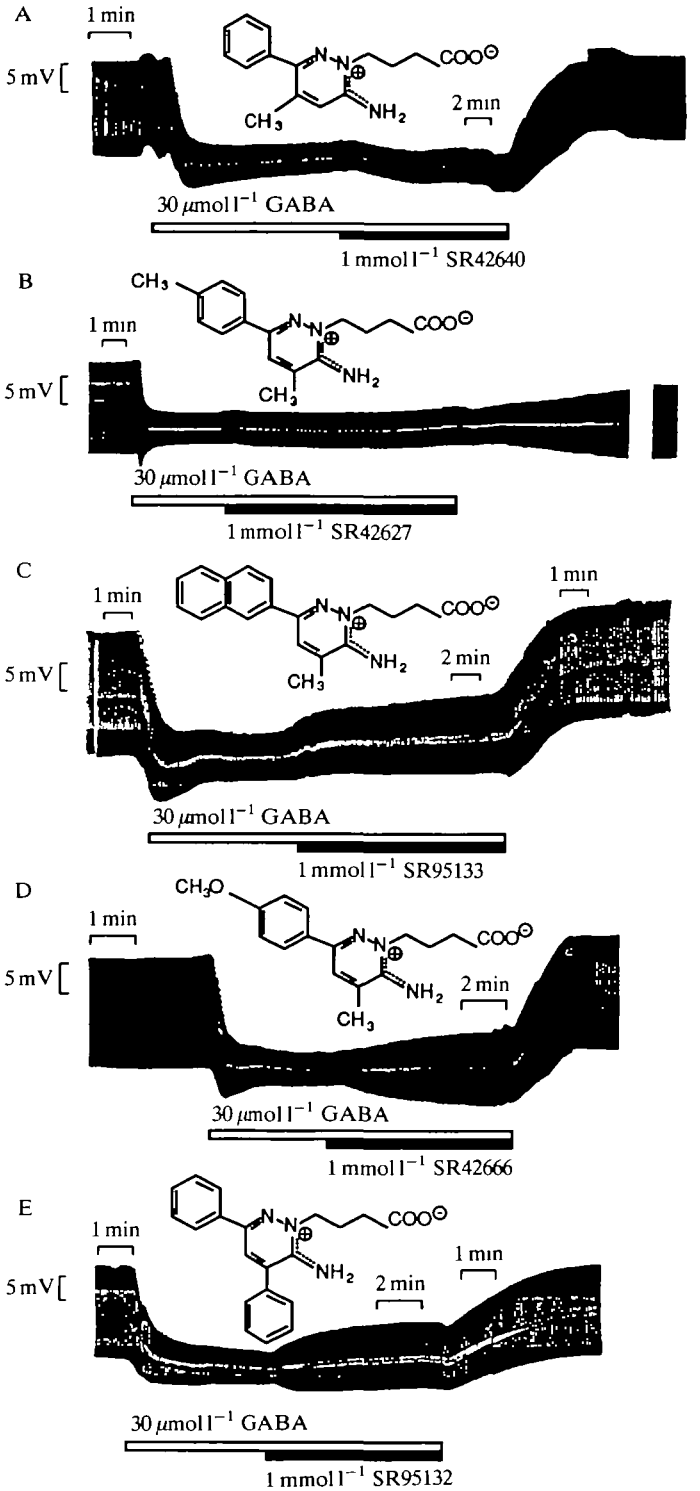
A
1 min
5 mV
2 min
30 μmol l⁻¹ GABA
1 mmol l⁻¹ SR42640
B
1 min
5 mV
30 μmol l⁻¹ GABA
1 mmol l⁻¹ SR42627
C
1 min
1 min
5 mV
2 min
30 μmol l⁻¹ GABA
1 mmol l⁻¹ SR95133
D
1 min
5 mV
2 min
30 μmol l⁻¹ GABA
1 mmol l⁻¹ SR42666
E
1 min
1 min
5 mV
2 min
30 μmol l⁻¹ GABA
1 mmol l⁻¹ SR95132

Fig. 2

Fig. 2. Effects of 1 mmol l^{-1} SR42640, SR42627, SR95133, SR42666 and SR95132 on the change in membrane input conductance produced by 30 μmol l^{-1} GABA. (A) Effect of 1 mmol l^{-1} SR42640: resting input conductance, G_0=2.2 μS; 30 μmol l^{-1} GABA, ΔG=4.6 μS; 1 mmol l^{-1} SR42640 + 30 μmol l^{-1} GABA, ΔG=3.9 μS; antagonism=15 %. (B) Effect of 1 mmol l^{-1} SR42627: resting input conductance, G_0=2.2 μS; 30 μmol l^{-1} GABA, ΔG=4.4 μS; 1 mmol l^{-1} SR42627 + 30 μmol l^{-1} GABA, ΔG=3.1 μS; antagonism=29 %. (C) Effect of 1 mmol l^{-1} SR95133: resting input conductance, G_0=2.0 μS; 30 μmol l^{-1} GABA, ΔG=3.8 μS; 1 mmol l^{-1} SR95133 + 30 μmol l^{-1} GABA, ΔG=1.4 μS; antagonism=63 %. (D) Effect of 1 mmol l^{-1} SR42666: resting input conductance, G_0=2.2 μS; 30 μmol l^{-1} GABA, ΔG=5.0 μS; 1 mmol l^{-1} SR42666 + 30 μmol l^{-1} GABA, ΔG=1.0 μS; antagonism=80 %. (E) Effect of 1 mmol l^{-1} SR95132: resting input conductance, G_0=2.0 μS; 30 μmol l^{-1} GABA, ΔG=3.7 μS; 1 mmol l^{-1} SR95132 + 30 μmol l^{-1} GABA, ΔG=0.7 μS; antagonism= 81 %.

electrophysiological approach in *Ascaris* and ligand-binding techniques in vertebrates. However, the potency order found in *Ascaris* can be compared to the potency order found in vertebrates. The two potent analogues in vertebrates, SR95531 and SR42627, are weak antagonists in *Ascaris*. In contrast SR95132, virtually inactive in vertebrates, is one of the most potent analogues in *Ascaris*.

In vertebrates, arylaminopyridazine derivatives act as competitive antagonists where they displace [^{3}H]GABA and [^{3}H]muscimol from their binding sites (Chambon *et al.* 1985; Heaulme *et al.* 1986*a*,*b*) and decrease [^{3}H]diazepam binding stimulated by GABA (Heaulme *et al.* 1986*a*). It has previously been shown that, in *Ascaris*, SR95103 produces a parallel shift, to the right, in GABA dose–response curves without a decrease in the maximum response (Duittoz and Martin, 1991*a*). The dose-dependency was best described by the modified Schild equation (Williams *et al.* 1988), suggesting that the mechanism was competitive and that only one molecule of SR95103 interacted with the GABA receptor (Duittoz and Martin, 1991*a*). The actions of SR95132 and SR42666 on GABA cumulative dose–response curves were examined at 100, 300 and 1000 μmol l^{-1}. SR95132 and SR42666 produced a parallel displacement to the right in GABA dose–response curves, without a reduction in the maximal response, suggesting a competitive mechanism. Fig. 3 illustrates the antagonism produced by SR95132. Note that in the presence of 3 μmol l^{-1} GABA the membrane was slightly depolarized without any detectable change in the membrane conductance (Fig. 3A); depolarization of the membrane was occasionally seen after application of low GABA concentrations (A. H. Duittoz and R. J. Martin, unpublished observations). Del Castillo *et al.* (1964*b*) also noticed that low GABA concentrations produced small, but statistically significant, depolarizations of the membrane. Dose ratios were calculated for each cell, as the ratio of EC_{50} values estimated for the GABA dose–response curves obtained in the absence and the presence of antagonist. Fig. 4A shows the dose–response curves for the traces shown in Fig. 3. The dose-dependent antagonism produced by SR42666 is illustrated in Fig. 5, and corresponding dose–response curves are illustrated in Fig. 6A. In Fig. 6A, ΔG_{max}

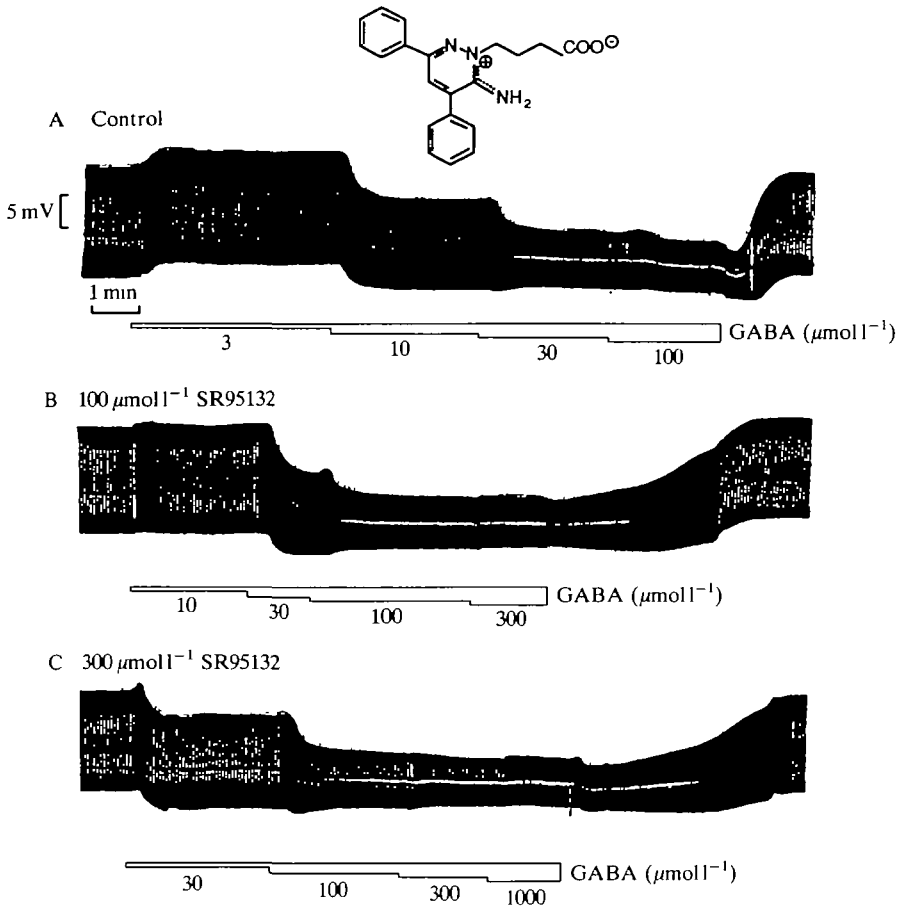

Fig. 3. Effects of 100 and 300 μmol l^{-1} SR95132 on the GABA dose–response relationship. (A) Control responses to cumulative application of GABA. Note the small depolarization observed with 3 μmol l^{-1} GABA where there was no detectable change in membrane input conductance. (B) The effect of 100 μmol l^{-1} SR95132. (C) The effect of 300 μmol l^{-1} SR95132. The corresponding dose–response curves are illustrated in Fig. 4. All three recordings were obtained from the same cell.

appears to be reduced in the presence of SR42666. This illustrates the difficulty in measuring high membrane conductance accurately. Errors made on ΔG measurements increase when the resistance decreases, so that the error in ΔG_{max} is large compared to the error in ΔG produced by lower concentrations of agonists. However, when results obtained from several cells are pooled, ΔG_{max} is not significantly affected by SR95103 (Duittoz and Martin, 1991*a*), SR42666 or SR95132.

The classical Schild plots for SR95132 (Fig. 4B) and SR42666 (Fig. 6B), log(DR−1) against log[X_B], were linear but with a slope different from 1: 0.73 for SR42666 (N=13) and 0.74 for SR95132 (N=9). However, the classical Schild plo

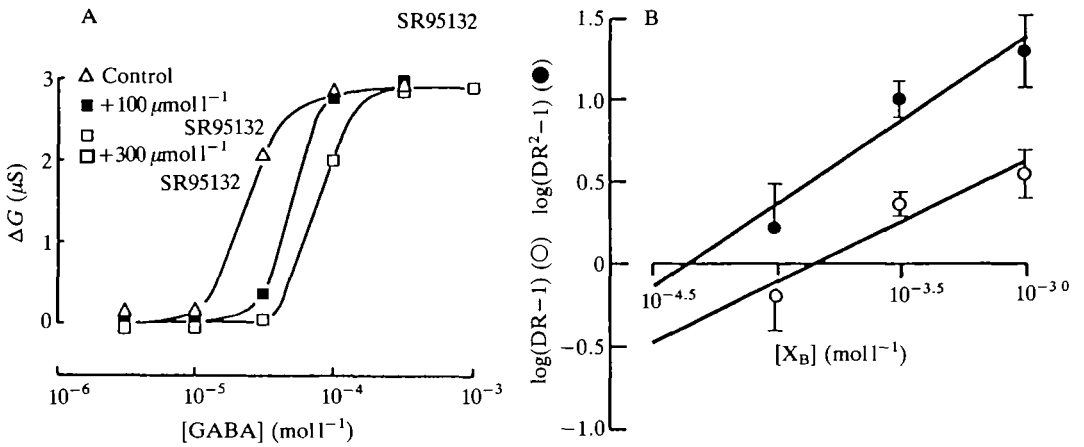

Fig. 4. Dose-dependency of the antagonism produced by SR95132. The lines correspond to the fitted modified Hill equation, whereas the points are the experimental data. (A) Dose–response curves for GABA. Control GABA dose–response curve (△); values estimated for the modified Hill equation are ΔG_{max}=2.8 μS, EC_{50}= 19.8 μmol l^{-1}, n=2.6. Dose–response curve for GABA in the presence of 100 μmol l^{-1} SR95132 (■), values estimated for the modified Hill equation are: ΔG_{max}=2.8 μS, EC_{50}=37.6 μmol l^{-1} and n=3.2. Dose–response curve for GABA in the presence of 300 μmol l^{-1} SR95132 (□), values estimated for the modified Hill equation are: ΔG_{max}=2.8 μS, EC_{50}=77.2 μmol l^{-1} and n=3.5. (B) Schild and modified Schild plots; (○) Schild plot, log(DR−1)/log[X_B], where [X_B] is the concentration of antagonist. Correlation coefficient, r=0.71; slope=0.74±0.25, P=0.02 (F-test); R^2=0.62. (●) Modified Schild plot, log(DR^2−1)/log[X_B]. Correlation coefficient r=0.75; slope=1.06±.32, P=0.01, (F-test); R^2=0.66, suggesting a better fit than with the classical Schild plot. Values are mean±S.E., N=9.

does not account for a stoichiometry greater than 1 between GABA and its receptor. The modified Schild equation was therefore used (Williams *et al.* 1988), and log(DR^2−1) was plotted against log[X_B] (Figs 4B and 6B). This plot yielded a better fit for SR95132 (R^2=0.66 against R^2=0.62 for the Schild plot). The linear fit was not improved for SR42666 (R^2=0.62), but, for both compounds, the slopes of the modified Schild plots were close to 1: 1.02 for SR42666 (N=13) and 1.06 for SR95132 (N=9). The interpretation of these linear regressions is limited, especially in terms of the goodness of fit, since only three concentrations of antagonist were used. A wider range of concentrations should be used for more reliable information. However, the use of higher concentrations of SR95132 and SR42666 was limited by the solubility of these compounds. Despite this, we believe that the use of the modified Schild analysis, rather than the classical one, is justified. The best argument is given by the strong evidence for bimolecular interaction between GABA and its receptor found in *Ascaris* (Holden-Dye *et al.* 1988) and more generally in vertebrate and invertebrate preparations (see Nistri and Constanti, 1979, for a review).

The results of the modified Schild analysis are apparently consistent with a

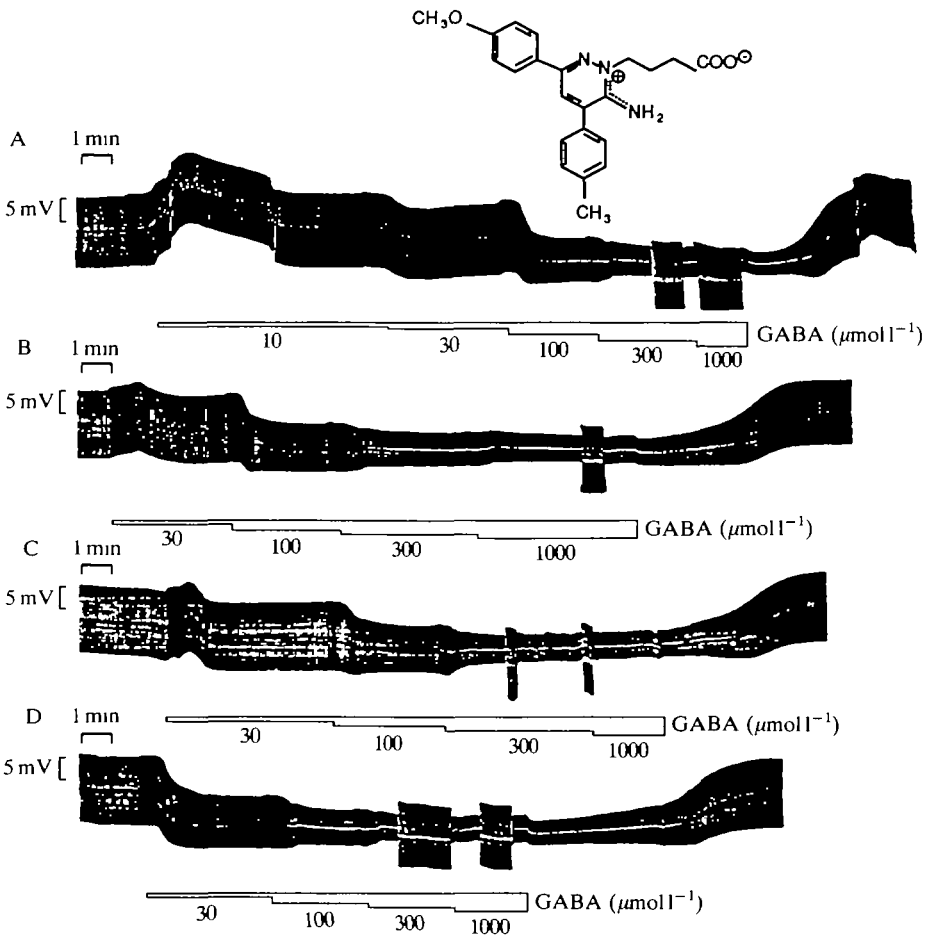

Fig. 5. Effects of 100, 300 and 1000 μmol l^{-1} SR42666 on the GABA dose–response relationship. (A) Control responses to cumulative application of GABA, G_0=2.2 μS. Note the transient depolarizing response in the presence of 10 μmol l^{-1} GABA and the temporary increase in gain from 5 mV to 2 mV per division during the application of 300 and 1000 μmol l^{-1} GABA. (B) In the presence of 100 μmol l^{-1} SR42666. Note the temporary increase in gain from 5 mV to 2 mV per division during the application of 1000 μmol l^{-1} GABA. (C) In the presence of 300 μmol l^{-1} SR42666. Note the temporary increase in gain from 5 mV to 2 mV per division during the application of 300 and 1000 μmol l^{-1} GABA. (D) In the presence of 1000 μmol l^{-1} SR42666. Note the gain changes in the presence of 300 and 1000 μmol l^{-1} GABA. The corresponding dose–response curves are illustrated in Fig. 6.

model that involves the activation of the receptor by two molecules of GABA but with only one molecule of SR42666 or SR95132 interacting with the receptor to block the effect. This is in agreement with previous results obtained with SR95103 (Duittoz and Martin, 1991*a*,*b*). Although these results do not establish such a mode of action, they provide a good quantitative description for the interaction

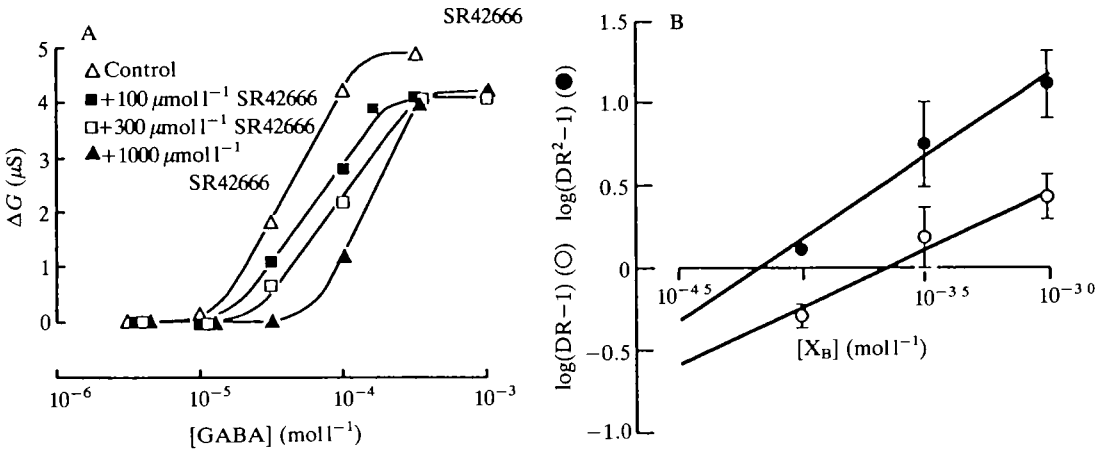

Fig. 6. Dose-dependency of the antagonism produced by SR42666. The lines correspond to the fitted modified Hill equation, whereas the points are the experimental data. (A) Dose–response curves for GABA. Control GABA dose-response curve (△); values estimated for the modified Hill equation are ΔG_{max}=4.9 μS, EC_{50}= 39.9 μmol l^{-1}, n=2.1. GABA dose–response curve in the presence of 100 μmol l^{-1} SR42666 (■), values estimated for the modified Hill equation are ΔG_{max}=4.1 μS, EC_{50}=59.8 μmol l^{-1} and n=1.7. GABA dose–response curve in the presence of 300 μmol l^{-1} SR42666 (□), values estimated for the modified Hill equation are ΔG_{max}=4.3 μS, EC_{50}=89.7 μmol l^{-1} and n=1.8. GABA dose–response curve in the presence of 1000 μmol l^{-1} SR42666 (▲), values estimated for the modified Hill equation are ΔG_{max}=4.3 μS, EC_{50}=134.4 μmol l^{-1} and n=3.2. In this particular cell ΔG_{max} appears to be smaller in the presence of SR42666. However, in other cells, ΔG_{max} can be higher in the presence of SR42666 than for the control. This illustrates the difficulty in measuring high membrane conductance accurately. SR42666 and the other arylaminopyridazine GABA derivatives tested quantitatively did not affect ΔG_{max} or n significantly. (B) Schild and modified Schild plots; (○) Schild plot, log(DR−1)/log[X_B], where X_B is the concentration of antagonist. Correlation coefficient, r=0.78; slope=0.73±0.17, P=0.001 (F-test); R^2=0.62. (●) Modified Schild plot, log(DR^2−1)/log[X_B]. Correlation coefficient r=0.78; slope=1.02±.25, P=0.001, (F-test); R^2=0.62. Values are mean±S.E., N=13.

between antagonist and receptor, and allow the estimation of apparent dissociation constants. The dissociation constant, K_B, was estimated for the antagonists, assuming n=2 and M=1:

$$K_B = (DR^2 - 1)/K_B .$$

For SR42666, K_B=104.8±19.7 μmol l^{-1} (N=13); and for SR95132, K_B= 65.0±20.3 μmol l^{-1} (N=9). SR95132 therefore appears equipotent to SR95103 (K_B=63.8±12.9 μmol l^{-1}, N=14, Duittoz and Martin, 1991*a*), whereas SR42666 is definitely less potent than SR95103 (t-test, $P \leq 0.05$). Since arylaminopyridazine GABA derivatives seem to act as competitive antagonist at the *Ascaris* GABA receptor, as they do at the vertebrate $GABA_A$ receptor, it is of interest to compare and discuss their respective structure–activity relationships.

Discussion

The measurement of the percentage of antagonism can be compared to a snapshot of the antagonist–receptor interaction, since only one concentration of antagonist and agonist are tested together. For that reason, it does not provide any information about the type of antagonism involved or any detailed quantitative information. However, within a family of compounds supposed to act in a similar manner, such as the arylaminopyridazine derivatives of GABA, the percentage of antagonism can be used to determine their potency sequence. The method is, nonetheless, a sensitive technique for detecting antagonism because the GABA concentration used, $30\,\mu mol\,l^{-1}$, is near to the EC_{50} for GABA and, therefore, in the middle of the linear part of the log dose–response curve.

When applied at $1\,mmol\,l^{-1}$ in the presence of $30\,\mu mol\,l^{-1}$ GABA, SR42627, SR42640, SR95133, SR95132, SR95103 and SR95531 present a potency order different from that observed at the vertebrate $GABA_A$ receptor (Wermuth *et al.* 1987). At the *Ascaris* GABA receptor the observed potency order is: SR95103≈ SR95132>SR42666>SR95133>SR95531>SR42627>SR42640, whereas, at the mammalian $GABA_A$ receptor the potency order is: SR95531>SR42666> SR42627>SR95103>SR95133>SR42640>SR95132.

In *Ascaris*, the three most potent analogues, SR95103, SR95132 and SR42666, have been shown to displace GABA dose–response curves to the right without a decrease in the maximal response. The modified Schild analysis (Williams *et al.* 1988), which accounts for the positive cooperativity between GABA and its receptor, suggested a competitive mechanism in which only one molecule of the antagonist was required to block the effects produced by two molecules of GABA. In conclusion, on the basis of the results obtained with SR95103, SR95132 and SR42666, the mode of action of arylaminopyridazine derivatives of GABA in *Ascaris* is considered to be competitive. The difference in relative potencies of arylaminopyridazine GABA derivatives at the vertebrate and the *Ascaris* GABA receptors provides evidence for different structural requirements. The GABA-like moiety of the arylaminopyridazine derivatives, supposed to interact with the GABA recognition site on the GABA receptor, is the same for all the analogues tested here. The difference in relative potencies seen in *Ascaris* suggests that different parts of the molecule might be involved. The structure–activity relationships of pyridazine derivatives have been investigated in great details in the vertebrate nervous system (Wermuth *et al.* 1987) and discussed in terms of Ariëns' theory of accessory binding sites (Ariëns *et al.* 1979). In this theory, the substituents on the pyridazine ring play an important role in the binding of the antagonist to the receptor and in its potency.

In vertebrates, a very important role is played by the substituent in the 6-position (Wermuth *et al.* 1987). In the present study, all the analogues tested had a phenyl, substituted or not, in the 6-position on the pyridazine ring, except SR95133 (α-napthyl). The role played by the 6-substituent has been associated with the existence of additional binding interactions with the receptor, involving π electrons (Wermuth *et al.* 1987). The napthyl 6-substituent found in SR95133 also

presents π electrons for interaction with the receptor, and this may explain why SR95133 retains some antagonist properties.

The substituent effects on the 6-phenyl group can influence the potency. For example, substituents increasing the electron density ($-CH_3$, as in SR42627) on the aromatic system ($-OCH_3$, as in SR95531), increase the potency in mammalian preparations, in contrast to electron-withdrawing substituents (Wermuth *et al.* 1987). In vertebrates, the order of potency for the 6-phenyl substituents is $-OCH_3$>$-CH_3$>-H (SR42666>SR42627>SR95103), and in *Ascaris* it is -H> $-OCH_3$>$-CH_3$ (SR95103>SR42666>SR42627), suggesting an opposite effect. However, in *Ascaris*, the 6-phenyl substituent is not the limiting factor for the antagonist properties, the presence of a 4-substituent on the pyridazine ring is more important.

The role played by the 4-substituent in *Ascaris* is illustrated by the potent antagonists in *Ascaris*, SR95103, SR42666 and SR95132, which are all substituted on the 4-position of the pyridazine ring. This contrasts with the vertebrate preparations, where the substitution by a methyl or a phenyl group in the 4-position on the pyridazine ring was always detrimental to the antagonist potency (Wermuth *et al.* 1987). The order of potency for the 4-substituent is -H>$-CH_3 \gg -C_6H_5$ (SR95531>SR95103>SR95132). By comparison, in *Ascaris*, it is $-C_6H_5 \approx -CH_3 \gg$-H (SR95132≈SR95103≫SR95531). To illustrate the importance of the 4-substituent in *Ascaris*, one can compare the potencies of SR42666 and SR95531: their chemical structure is identical except for the 4-methyl present in SR42666 but not in SR95531. SR42666 is more potent than SR95531 in *Ascaris*. This contrasts with mammalian preparations where SR95531 is more potent than SR42666. The presence of a 4-substituent appears to be a prerequisite for the antagonist potency of pyridazine GABA derivatives in *Ascaris*, whereas the opposite situation is found in vertebrates.

In vertebrates, computer graphic modelling of the pyridazinyl GABA derivatives and other known GABA antagonists was used to illustrate a plausible model for the interaction of competitive antagonists with the $GABA_A$ receptor (Wermuth and Rognan, 1987). The superimposition of various $GABA_A$ antagonists shows the existence of an anionic and a cationic system separated by a distance similar to that found in the GABA molecule (Wermuth and Rognan, 1987). The antagonist properties were explained by the presence of additional binding sites corresponding to the various aromatic or aliphatic rings of these compounds. Fig. 7 shows the superimposition of SR95531 and SR95132; the lack of activity of SR95132 on the vertebrate $GABA_A$ receptor was explained by the obstruction of the free access zone required for receptor recognition by the phenyl group in the 4-position (Wermuth and Rognan, 1987). Obviously this is not the case in *Ascaris*, where the presence of a substituent in the 4-position is apparently a requirement for the antagonistic activity.

The existence of different accessory binding sites responsible for antagonist properties in *Ascaris* would explain the weak activity of the classical competitive $GABA_A$ antagonists, such as bicuculline, pitrazepine, securinine (Holden-Dye

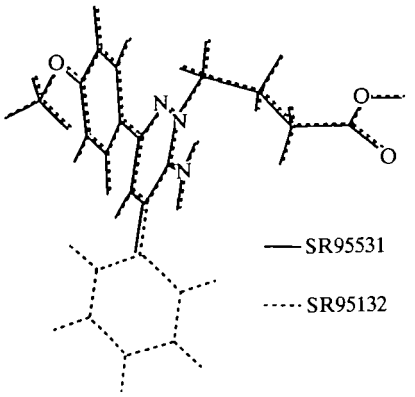

Fig. 7. Superimposition of SR95132 and SR95531. The superimposition of these two analogues shows that the 4-substituent is found in the proximity of the GABA recognition site. The inactivity of 4-substituted analogues at the vertebrate $GABA_A$ receptor was explained in terms of restriction of the free access zone required for interaction with the receptor. The volume occupied by the phenyl group of SR95132 is visualized. (Reproduced from Wermuth and Rognan, 1987, with permission.)

et al. 1988), tubocurarine and RU5135 (A. H. Duittoz and R. J. Martin, unpublished observations). The presence of alternative binding sites is not inconsistent with arylaminopyridazine derivatives acting as competitive antagonists, since the binding of the GABA-like moiety may be essential. In mammalian preparations, replacement of the GABA-like moiety by propionic, valeric or caproic acid analogues decreases the potency, although it does not lead to a complete loss of antagonistic activity (Wermuth *et al.* 1987). This suggests that the flexible side chain plays a role in the antagonist potency, but that a strict resemblance to GABA is not necessary. The existence of accessory binding sites on the GABA receptor is not the only possible explanation. For example, substituents on the pyridazine ring may influence the affinity of the antagonist for the GABA binding site, without binding to other parts of the receptor. In this case, the difference in relative potency seen between vertebrates and *Ascaris* would be explained by differences in the GABA binding site itself. However, agonist studies in *Ascaris* (Holden-Dye *et al.* 1989) have shown that the *Ascaris* muscle GABA receptor and the vertebrate $GABA_A$ receptor are correlated in terms of agonists properties, suggesting some structural similarities between the *Ascaris* and vertebrate GABA recognition sites. The hypothesis of different accessory binding sites on the *Ascaris* GABA receptor is, therefore, the most attractive one. Only a study of the molecular stucture of this receptor and its comparison to the structure of the vertebrate $GABA_A$ receptor would bring direct evidence for these putative sites.

These differences are obviously of great interest in the search for new anthelmintic drugs, and could lead to the synthesis of potent GABA antagonists to *Ascaris* GABA receptors, which are inactive in mammals and, therefore, without

toxicity or secondary effects in the host. Further characterization of the structure–activity relationships of pyridazine GABA derivatives may give rise to interesting developments in the future.

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