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GABA receptors on the somatic muscle cells of the parasitic nematode, *Ascaris suum*: stereoselectivity indicates similarity to a $GABA_A$-type agonist recognition site

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1 The γ-aminobutyric acid (GABA) receptors on the somatic muscle cells of *Ascaris*, which mediate muscle cell hyperpolarization and relaxation, have been characterized by use of intracellular recording techniques.

2 These receptors are like mammalian $GABA_A$-receptors in that the response is mediated by an increase conductance to chloride ions. The $GABA_A$-mimetic, muscimol, has a relative potency of 0.40 ± 0.02 ($n = 3$) compared to GABA.

3 The stereoselectivity of the GABA receptor on *Ascaris* is identical to that for the mammalian $GABA_A$-receptor, as determined from the relative potency of three pairs of enantiomers of structural analogues of GABA.

4 The most potent agonist is (S)-(+)-dihydromuscimol which is 7.53 ± 0.98 ($n = 5$) times more potent than GABA.

5 The *Ascaris* GABA receptor is not significantly blocked, at concentrations below 100 μM by the potent, competitive $GABA_A$-receptor antagonist, SR95531.

6 The *Ascaris* GABA receptor does not recognise agents that are known to block the GABA gated chloride channel in mammalian preparations such as *t*-butylbicyclophosphorothionate (TBPS, 10 μM, $n = 2$) or the insecticide dieldrin (100 μM, $n = 3$).

7 GABAergic responses in *Ascaris* are not potentiated by pentobarbitone (100 μM, $n = 3$) or flurazepam (100 μM, $n = 3$).

8 The potencies of various GABA-mimetics in the *Ascaris* preparation have been compared with their potency at displacing $GABA_A$-receptor binding in mammalian brain. Excluding the sulphonic acid derivatives of GABA, the correlation coefficient (r) between the potencies of compounds in the two systems is 0.74 ($P < 0.01$). The significance of this correlation is discussed.

9 The pharmacology of the *Ascaris* GABA receptor is discussed in relation to other invertebrate systems and the mammalian subclassification of GABA receptors.

Introduction

Invertebrate γ-aminobutyric acid (GABA) receptors do not readily fit into the mammalian subclassification of $GABA_A$- and $GABA_B$-subtypes (see Walker & Holden-Dye, 1989 for review) as described for the mammalian system by Bowery *et al.*, 1980. Thus, whilst many of them are similar to the mammalian $GABA_A$-receptor subtype in that both responses are mediated by the opening of a chloride ionophore (Fatt & Katz, 1953; Boistel & Fatt, 1958; Takeuchi & Takeuchi, 1967; Motokizawa *et al.*, 1969; Pitman & Kerkut, 1970; Curtis & Johnston, 1974; Krnjević, 1974; Bokisch & Walker, 1986; Pinnock *et al.*, 1988), they are consistently insensitive or only very weakly activated by the sulphonic acid

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GABA analogues, such as 3-aminopropane sulphonic acid or piperidine-4-sulphonic acid in insect (Roberts *et al*., 1981; Sattelle *et al*., 1988), arthropod (Roberts *et al*., 1981), gastropod (Vehovsky *et al*., 1989), and nematode (Holden-Dye *et al*., 1988) preparations. This is in marked contrast to the potent agonist action of these compounds at $GABA_A$-receptors in mammalian and amphibian preparations (Curtis & Watkins, 1961; Campochiaro *et al*., 1977; Nistri & Constanti, 1979).

Another difference between invertebrate and mammalian $GABA_A$-receptors is that apart from a few exceptions (Walker *et al*., 1971; Piggott *et al*., 1977; Yarowsky & Carpenter, 1978; Waldrop *et al*., 1987), these receptors are generally only weakly (Smart & Constanti 1986), or not blocked (Scott & Duce 1987; Sattelle *et al*., 1988; Benson 1988a; Holden-Dye *et al*., 1988) by the definitive, competitive $GABA_A$-receptor antagonist, bicuculline. Nevertheless, micromolar concentrations of the noncompetitive antagonist picrotoxin, which acts at the level of the chloride channel in mammalian preparations (Olsen, 1981; Barker, 1985), is sufficient to block GABA-mediated responses in most invertebrates (Piggott *et al*., 1977; Hori *et al*., 1978; Constanti, 1978; Walker & Roberts, 1982; Sattelle *et al*., 1988). In arthropods the interaction of picrotoxin with the receptor channel complex may differ slightly from the interaction in mammalian systems in that the block may not be truly noncompetitive (Constanti, 1978).

$GABA_A$-mediated responses in mammalian preparations are potentiated by benzodiazepines and barbiturates (Olsen, 1981 for review). There is some evidence for such an interaction at insect GABA receptors (Beadle *et al*., 1986; Robinson *et al*., 1986; Lees *et al*., 1987). Whether or not other invertebrate GABA systems will also exhibit such modulation has yet to be determined.

The GABA receptor described in this study mediates muscle cell hyperpolarization and relaxation in the parasitic nematode *Ascaris suum*. GABA is apparently the endogenous ligand for this receptor as, using GABA-specific antisera, GABA-immunoreactivity has been localized in the inhibitory motorneurones and activation of these neurones elicits inhibitory postsynaptic potentials in the muscle cells (Johnson & Stretton, 1987). Other inhibitory amino acids, (glycine, β-alanine and taurine) are without effect on the *Ascaris* muscle cell (Holden-Dye *et al*., 1988). The GABA receptor on *Ascaris* muscle cells is neither bicuculline- nor picrotoxin-sensitive (Wann, 1987; Holden-Dye *et al*., 1988). Studies using various GABA receptor agonists, however, have indicated that the agonist recognition site may resemble the mammalian $GABA_A$-receptor in some respects. Thus, it is activated by isoguvacine and muscimol (Holden-Dye *et al*., 1988). A further similarity is indicated by the observation that the agonists show positive cooperativity at the *Ascaris* GABA receptor (Holden-Dye *et al*., 1988), as they do at receptors in mammalian preparations (Nistri & Constanti, 1979). Furthermore, although the GABA response is not blocked by picrotoxin, which is known to act at the GABA-operated anion channel in mammalian preparations, the response to GABA in *Ascaris* muscle is accompanied by an increase in conductance of the membrane for chloride ions (Martin 1980; Holden-Dye & Walker, 1989).

The *Ascaris* GABA receptor is not sensitive to the $GABA_B$-receptor agonist, baclofen (Holden-Dye *et al*., 1988). Indeed, there is no evidence to date of a $GABA_B$-type receptor in invertebrate preparations.

In this study we have further characterized the *Ascaris* GABA receptor. We have tested for antagonism by a potent, competitive $GABA_A$-antagonist SR95531, by noncompetitive $GABA_A$-receptor antagonists and for modulation by known modulators of GABA-receptors in mammalian preparations. In particular, we have used stereoisomers of structural analogues of GABA to test more rigorously the notion that the agonist recognition site at GABA receptors in the invertebrate *Ascaris* resembles that of the mammalian $GABA_A$-receptor.

Methods

Ascaris were obtained from the local abattoir on a weekly basis and maintained in the laboratory. An anterior section of the worm, approximately 2 cm long, was excised and slit along one lateral line to enable the strip to be laid out flat. The intestine was removed revealing the muscle bag cells. The section was securely pinned out, cuticle side down, in a perspex perfusion chamber and continuously perfused with artificial perienteric fluid (APF), 32–34°C, the composition of which was as follows: (mM) NaCl 67, Na acetate 67, KCl 3, $CaCl_2$ 3, $MgCl_2$ 15.7, Tris base 5, pH 7.6 with acetic acid. The cells were impaled with two potassium acetate (4 M)-filled glass microelectrodes with a resistance of 10–30 MΩ. Conventional electrophysiological recording techniques were employed using an Axoclamp 2A system. Transmembrane potentials were monitored. Input conductance of the cells was estimated by injecting current pulses, (0.1 Hz, 500 ms pulse width, 10–35 nA) through the second microelectrode and recording the resulting electrotonic potentials. Generally speaking there is little rectification in the hyperpolarizing direction so this method provides an estimate of the input conductance (Figure 1). Drugs were applied via a fine bore plastic tube (0.8 mm internal diameter)

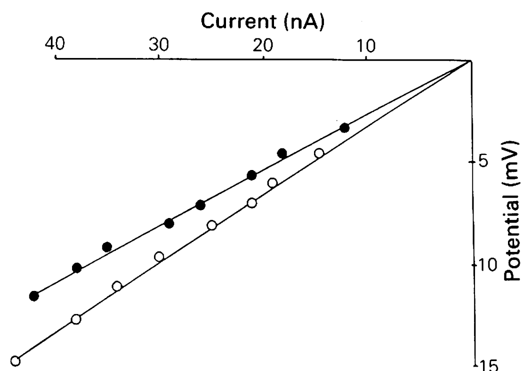

Figure 1 The current-voltage relationship for an *Ascaris* muscle cell. The cell was impaled with two microelectrodes. Hyperpolarizing current pulses, 10–40 nA, 500 ms pulse width, 0.1 Hz, were passed through one microelectrode and the electrotonic potential in the cell was monitored with the second electrode. The plots in the absence (○) and presence (●) of GABA are linear indicating that the cell did not rectify in the hyperpolarizing direction.

directed at the cell from which a recording was being made. Drug solutions were separated by air bubbles in the perfusion tubing which escaped through a small hole in the tubing immediately prior to entry to the bath. This method of application allows rapid and complete change of the composition of the perfusion stream over the cell under study (Slater & Carpenter, 1984). Studies with dye indicate that the perfusion stream bathes the cell under study and then swirls around the chamber. The dye is apparently evenly distributed in the perfusion chamber after 40 s. Drugs were applied at a rate of 8–10 ml min^{-1} for a period of up to 45 s (enabling the maximum response to develop) followed by a 5 min wash. Each drug was applied in ascending concentrations. With this technique, the conductance increase elicited by a given concentration of GABA is stable to multiple applications. Relative potencies of compounds compared to GABA were determined by fitting the dose-response curves by eye and taking the ratio of the concentration of the drug to the concentration of GABA that elicit equivalent responses in the same cell from a parallel portion of the graphs. Statistical significance was determined by Student's paired *t* test. Linear regression analysis was performed using the Biosoft programme, LINEFIT (Barlow, 1983). Maximum responses and EC_{50} values were obtained by fitting the dose-response data to saturation curves using the programme Fit&Hill (Barlow, 1983). The stereoisomers of the structural GABA analogues, (S)-(−)-4-methyl-trans-aminocrotonic acid (4-me-TACA), (R)-(+)-4-me-TACA, (S)-(+)-3-OH-GABA, (R)-(−)-3-OH-GABA, (S)-(+)-dihydromuscimol (DHM) and (R)-(−)-DHM were prepared as described previously (Krogsgaard-Larsen *et al.*, 1985). GABA was obtained from BDH, *t*-butylbicyclophosphorothionate (TBPS) from Research Biochemicals Inc., flurazepam from Roussel Laboratories. We are grateful to Q. Sanofi Recherche for the gift of SR95531 (2-[carboxy-3'-propyl]-3-amino-6-paramethoxy-phenyl-pyridazinium-Br). Water insoluble compounds such as dieldrin were dissolved in dimethylsulphoxide (DMSO) and diluted in artificial perienteric fluid (APF) such that the final concentration of DMSO was 1%. Other drugs and chemicals were supplied by Sigma.

Results

The resting membrane potential of the *Ascaris* muscle cells was 29.6 ± 0.6 mV with a resting input conductance of 2.53 ± 0.15 μS ($n = 28$). The responses obtained to GABA agree well with those that we have found previously (Holden-Dye *et al.*, 1988). The *Ascaris* muscle cells responded reproducibly to GABA with the threshold for the hyperpolarization between 5–10 μM GABA and the maximum response, of between 10–20 mV depending upon the resting membrane potential, developing at around 30 μM GABA (Figure 2). The maximum conductance change varies between cells in the range 1–3 μS, however the dose-response relationship is consistent, with an EC_{50} of 26.3 ± 3.5 μM ($n = 17$), as determined from 17 cells in which a clear maximum was obtained. Uptake is unlikely to affect significantly the potency of GABA or GABAmimetics in this preparation as it has been shown previously that the GABA uptake inhibitor, nipecotic acid, does not affect the potency of GABA (Hewitt, 1987). This does not necessarily mean that an uptake mechanism does not exist for GABA in these cells. It is most likely that the perfusion rate would overcome the effects of an uptake system in this study. In three paired experiments the relative potency of muscimol compared to GABA was determined (Figure 3). The mean relative potency compared to GABA was 0.40 ± 0.02 ($n = 3$). The slope of the dose-response curve is very similar to that for GABA indicating that muscimol interacts with the receptor in a similar manner to GABA, i.e. it exhibits positive cooperativity.

SR95531 did not significantly block the GABA response at 100 μM (Figure 4), although at 500 μM about 40% blockade was evident. This effect was completely reversible.

Dieldrin (100 μM) did not block the conductance increase elicited by GABA in *Ascaris* muscle cells (Figure 5). This agent was applied at least 10 min

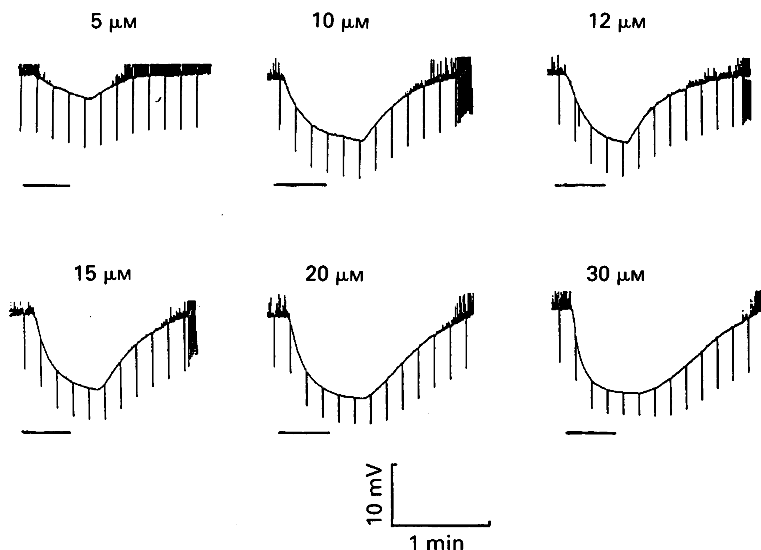

Figure 2 Hyperpolarization and increase in input conductance of the *Ascaris* somatic muscle cell to locally applied GABA. The duration of GABA application is indicated by the bar. The traces illustrate the responses obtained to increasing concentrations of GABA (as indicated). The resting membrane potential of this cell was −31 mV, resting input conductance 2.5 μS. Downward deflections are the electrotonic potentials resulting from the injection of 20 nA, 0.1 Hz, 500 ms pulse width.

before the addition of GABA and during the GABA application.

There was no evidence for a significant enhancement of the GABA response by either pentobarbitone (100 μM) or by flurazepam (100 μM; Figure 6). In one experiment the cell was exposed to 100 μM flurazepam for 60 min and the GABA response was not enhanced.

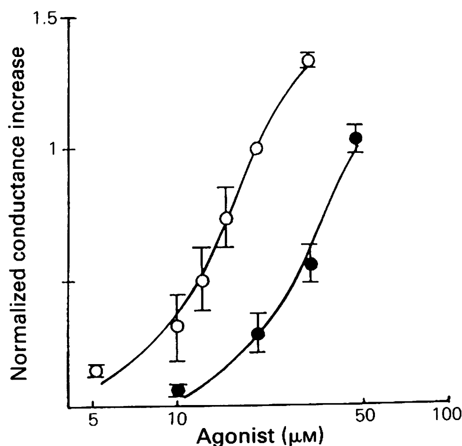

Figure 3 The increase in input conductance in *Ascaris* muscle cell evoked by bath applied GABA (○) and muscimol (●). The conductance increase was normalized with respect to the response obtained with 20 μM GABA ($n = 3$).

In a series of 5 experiments the relative potencies of the stereoisomers of the structural analogues of GABA compared to GABA were determined. All the compounds studied were GABAmimetic in that they elicited a membrane hyperpolarization and an increase in input conductance (Figure 7), though with varying degrees of potency (Table 1). The isomers of DHM and 3-OH-GABA were all apparently full agonists as shown by the fact that their dose-response curves are parallel to that for GABA (Figure 8). The isomers of 4-me-TACA may also be full agonists but their low potency meant that this could not be determined in this preparation. The degree of stereoselectivity for the compounds was most marked for the DHM compounds, with the (S)-(+)-isomer being nearly 10 times more potent than the (R)-(−)-isomer. (R)-(−)-3-OH-GABA was only 2 times more potent than the (S)-(+)-isomer of this compound; however, this difference in potency, though small was statistically significant ($P < 0.01$, $n = 5$, paired Student's t test) indicating that the receptor does truly distinguish between these compounds. (S)-(+)-DHM was by far the most potent compound at the GABA receptor in this preparation and is the most potent compound at this site that we have found to date. The (S)-(−)-isomer of 4-me-TACA was only weakly effective in eliciting a response, being approximately 20 times less potent than GABA. Threshold responses for the compound (R)-(+)-4-me-TACA were above 1 mM making

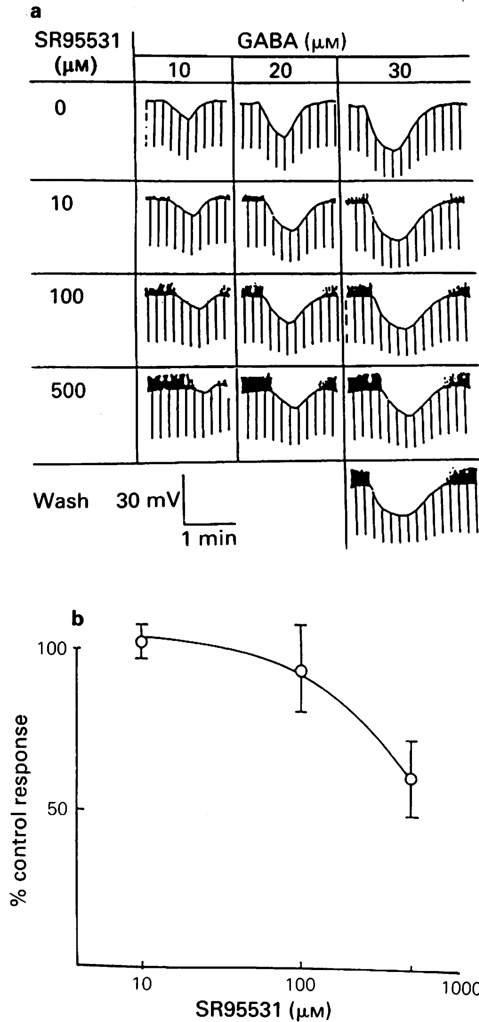

Figure 4 Weak antagonism of the GABA response by SR95531. (a) Typical responses to GABA (10, 20 and 30 μM) before and after the addition of SR95531. (SR95531 was applied by local perfusion for 2 min before the GABA application and with GABA). The downward deflections are elicited by injection of current (20 nA, 500 ms, 0.1 Hz) and allow determination of the input conductance. The resting membrane potential of this cell was -30 mV and the resting input conductance was 2.5 μS. Note that the GABA-induced increase in input conductance is only noticeably depressed at 500 μM SR95531. The wash was for 5 min and resulted in complete recovery of the conductance response to GABA. (b) The inhibition of the response to GABA by SR95531 in 3 separate preparations (with s.e.mean shown by vertical bars). The conductance increase to 30 μM GABA in the presence of SR95531 is expressed as a percentage of the conductance increase to 30 μM GABA in the absence of SR95531.

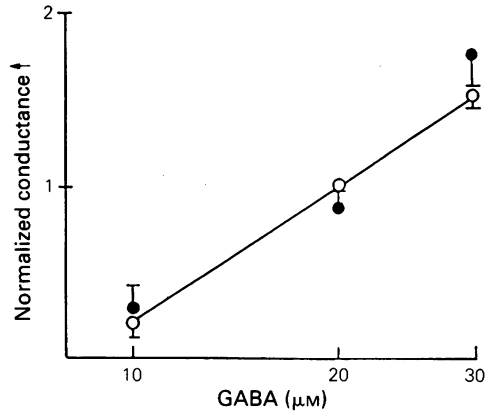

Figure 5 Inability of a non-competitive $GABA_A$-receptor antagonist to block the GABA response in *Ascaris*. Normalized conductance increase to GABA in the absence (○) and presence (●) of 100 μM dieldrin (n = 3, vertical bars show s.e.mean).

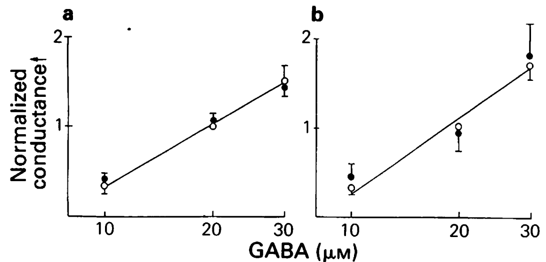

Figure 6 Lack of modulation of the GABA response by benzodiazepines and barbiturates. (a) Normalized conductance increase to GABA in the absence of (○) and after at least 10 min pre-exposure to (●) 100 μM pentobarbitone (n = 3, vertical bars show s.e.mean) (b) Normalized conductance increase to GABA in the absence (○) of and after at least 10 min pre-exposure to (●) 100 μM flurazepam (n = 3).

impossible an accurate determination of its relative potency compared to GABA.

Discussion and conclusions

The *Ascaris* muscle bag cells responded to locally applied GABA with an increase in membrane conductance accompanied by a hyperpolarization. This response was mimicked by the $GABA_A$-receptor agonist, muscimol. However, the receptor showed little similarity to the $GABA_A$-receptor in terms of blockade by competitive or noncompetitive antagonists, or in terms of modulation by benzodiazepines

Table 1 The potencies of structural analogues of γ-aminobutyric acid (GABA) at eliciting an increase in input conductance at the *Ascaris* GABA receptor were determined in 5 preparations: relative potencies of the compounds compared to GABA were determined from equipotent concentrations of the drug and GABA on parallel portions of the dose-response curve

Drug	*Relative potency*
(S)-(+)-dihydromuscimol	7.53 ± 0.98
GABA	1
(R)-(−)-dihydromuscimol	0.85 ± 0.08
(R)-(−)-3-OH-GABA	0.25 ± 0.02*
(S)-(+)-3-OH-GABA	0.13 ± 0.02
(S)-(−)-4-methyl-TACA	0.007 ± 0.001
(R)-(+)-4-methyl-TACA	No effect at 1 mM.

A relative potency of less than 1 indicates the compound is less potent than GABA (*$P < 0.01$ compared to (S)-(+)-3-OH-GABA). Each value is the mean ± s.e.mean, $n = 5$.

and barbiturates. We have previously reported a lack of sensitivity of the *Ascaris* GABA receptor to bicuculline and to other competitive $GABA_A$-receptor antagonists (Holden-Dye *et al*., 1988). Recently it has been suggested that the pyridazinyl-GABA derivative, SR95103, is a competitive antagonist at the *Ascaris* GABA receptor (Duittoz & Martin, 1989). This substance belongs to a class of compounds that were developed as selective, competitive, potent $GABA_A$-receptor antagonists (Wermuth & Bizière, 1986). In binding studies it was demonstrated that SR95103 and SR95331, another pyridazinyl-GABA derivative, were approximately 20 and 200 times more potent than bicuculline, respectively (Wermuth & Bizière, 1986). Similar results were noted in mammalian electrophysiological studies (Desarmenien *et al*., 1987). Thus, the lack of effect of SR95531, the most potent pyridazinyl derivative available, at the *Ascaris* GABA receptor unless high micromolar concentrations are used, indicates that this class of compounds does not recognise the *Ascaris* GABA receptor. TBPS and dieldrin are agents that are known to inhibit GABA-stimulated chloride flux in vertebrate preparations (Squires *et al*., 1983; Gant *et al*., 1987). Both are reasonably potent in these preparations with an effect occurring in the sub-micromolar range. Binding sites for TBPS, sensitive to dieldrin, have been identified in insect ganglia (Lunt *et al*., 1988). These compounds are believed to act in a noncompetitive manner, exerting their blocking action at the channel site rather than at the receptor. In *Ascaris*, however, neither of these agents had a

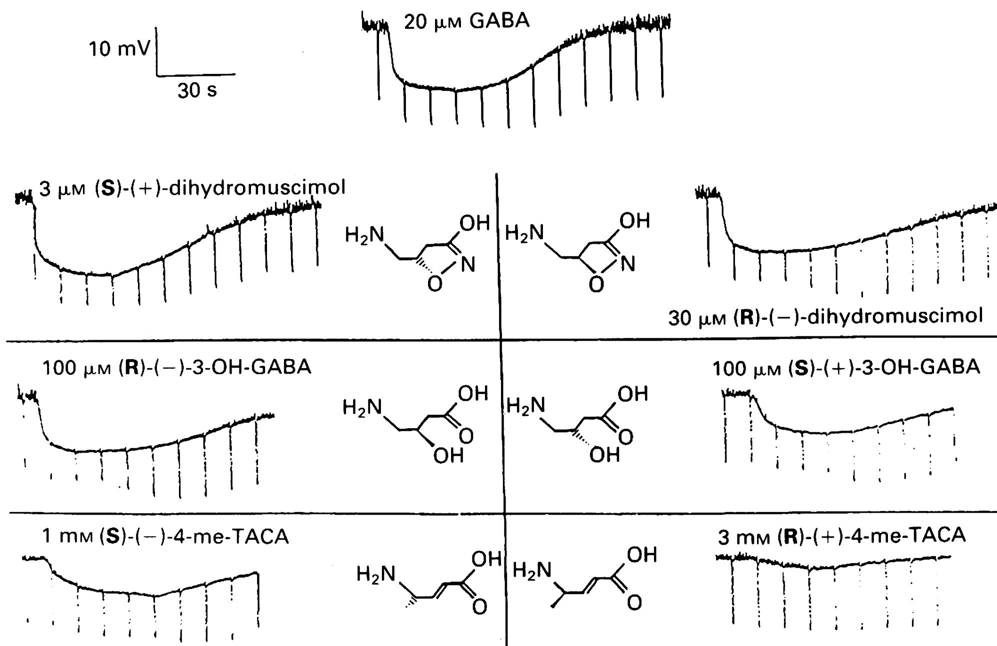

Figure 7 The GABA-mimetic activity of stereoisomers of structural analogues of GABA illustrating the structures of the compounds tested. The drugs were applied by local perfusion at the concentration indicated, for 30 s.

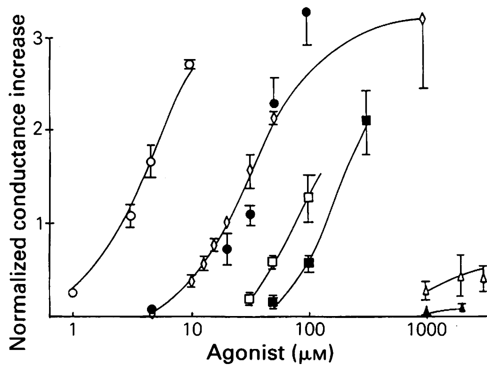

Figure 8 Dose-response relationships for stereoisomers of structural analogues of GABA. The conductance increases are normalized with respect to the response for 20 μM GABA. GABA (◇); (S)-(+)-DHM (○); (R)-(−)-DHM (●); (R)-(−)-3-OH-GABA (□); (S)-(+)-3-OH-GABA (■); (S)-(−)-4-me-TACA (△); (R)-(+)-4-me-TACA (▲). ($n = 3–6$, vertical bars show s.e.mean).

significant effect on the GABA response (Figure 5; Holden-Dye *et al.*, 1988). The tissue was pre-exposed to the compounds 10 min before the addition of GABA so it is unlikely that access was a problem. We could find no evidence that the *Ascaris* GABA receptor is coupled to either a benzodiazepine or a pentobarbitone modulatory site. In insects it has been found that 10 μM pentobarbitone or 10 μM flurazepam enhance the GABA response by up to 70% (Beadle *et al.*, 1986). Neither of these compounds had an effect in *Ascaris* at concentrations up to 100 μM (Figure 6). Thus, the GABA gated anion channel in *Ascaris* is pharmacologically distinct from that coupled to the mammalian $GABA_A$-receptor and also different from the GABA receptor in insect ganglia. The *Ascaris* GABA receptor is apparently novel in its lack of recognition of typical competitive and noncompetitive antagonists for $GABA_A$- receptors whilst being insensitive to baclofen and coupled to a chloride ionophore.

GABA, in common with a number of endogenous transmitter agents, is a flexible molecule able to assume a number of different conformations only one of which may be suitable for receptor activation (Krogsgaard-Larsen, 1988). The receptor in *Ascaris* is similar to other GABA receptors in preferring the extended conformation of GABA, corresponding to the compound trans-aminocrotonic acid (Holden-Dye *et al.*, 1988). The structural analogues of GABA that were tested in this study were GABA-mimetic and revealed further stereoselectivity at this GABA receptor. The degree of stereoselectivity seems to depend on the relative rigidity of the molecule. Thus, the most inflexible molecule, DHM, exhibits the greatest degree of stereoselectivity with the S-isomer being 10 times more potent than the **R**-isomer. There is only a slight degree of stereoselectivity between the enantiomers of 3-OH-GABA. Presumably, molecules such as 3-OH-GABA can overcome, to a certain extent, the steric hindrance imposed by the substitution of an unfavourable group by movement around the unfixed bonds. Both hydroxyl group substitution at the 3 position, and methyl group substitution at the 4 position decreased the efficacy of the compounds at the GABA receptor. The substitution of a methyl group seems to be particularly detrimental for GABA-mimetic activity at this site as 4-me-TACA was particularly weak at eliciting a response. The receptor, however, still distinguishes to a small extent between the two isomers of this compound. The stereoselectivity of the receptor for DHM and 4-me-TACA was the same as that found for the displacement of $GABA_A$-receptor binding in mammalian brain (Krogsgaard-Larsen *et al.*, 1988). The stereoselectivity for 3-OH-GABA, at the $GABA_A$-binding site (Krogsgaard-Larsen *et al.*, 1988) is the opposite of what we found in *Ascaris.* On spinal neurones, however, the (**R**)-(−)-enantiomer of 3-OH-GABA is marginally more potent than the (S)-(+)-enantiomer (Krogsgaard-Larsen *et al.*, 1985), corresponding exactly to the stereoselectivity that we observed at the *Ascaris* GABA receptor.

It has been established that the $GABA_A$-receptor not only has rigid requirements for the structural configuration of GABAergic compounds but also the degree of delocalization of both the positive and negative charges on the molecule is a crucial determinant of GABA-mimetic activity (Steward *et al.*, 1975). The most potent compound we have studied to date at the *Ascaris* muscle GABA receptor is DHM. This compound is also remarkably potent at gastropod neurones and at mammalian $GABA_A$-binding sites and neurones (Roberts *et al.*, 1981; Krogsgaard-Larsen *et al.*, 1985). The S-isomer of this compound is nearly 8 times more potent in *Ascaris* than GABA itself and on this basis we propose that it most accurately reflects the active structural and electronic configuration of GABA at the receptor. This molecule may provide a lead for the development of novel, competitive antagonists at the *Ascaris* GABA receptor if slight adjustments can be made to the molecule such that it retains its high affinity for the GABA receptor but its efficacy is decreased.

The results presented above clearly indicate that despite the lack of recognition of typical $GABA_A$-receptor antagonists by the receptor in *Ascaris* the receptor is sensitive to the $GABA_A$-agonist, muscimol. It has also previously been shown that the GABA-mimetic and anthel-

mintic agent piperazine acts as a weak GABA agonist in *Ascaris* (Martin, 1982). The sulphonic acid derivatives of GABA are without any effect in *Ascaris*. We have tested 3-aminopropane sulphonic acid and piperidine-4-sulphonic acid (P4S) both of which are without effect up to 1 mM (Holden-Dye *et al.*, 1988), despite the fact that these compounds have been shown to be extremely potent $GABA_A$-mimetic agents in electrophysiological studies in mammalian and amphibian preparations (Nistri & Constanti, 1979 for review). Sulphonic acid derivatives, however, are universally ineffective at GABA receptors in invertebrates and will not activate gastropod, arthropod or insect GABA receptors (see Introduction). In mammalian brain too, there is some circumstantial evidence to suggest that the way in which the receptor recognises the sulphonic acids and GABA differs. For example, the order of potency of compounds at displacing GABA binding is of the same relative order as that for displacing P4S binding, however the absolute potency is different by an order of magnitude (Krogsgaard-Larsen, 1981; Falch & Krogsgaard-Larsen, 1982). Furthermore, the sulphonic acids exhibit differences from GABA in the way in which they are coupled to the modulatory sites for barbiturates and benzodiazepines. P4S is a very weak stimulator of diazepam binding compared to GABA (Olsen, 1981; Olsen & Snowman, 1982; Krogsgaard-Larsen, 1988) and in contrast the binding of P4S is remarkably sensitive to stimulation by barbiturates (Olsen, 1981). On the basis of this evidence Krogsgaard-Larsen (1988) has suggested that sulphonic acid derivatives interact with the GABA receptor in a fundamentally different fashion from GABA itself.

The correlation between the ability of agents to stimulate the *Ascaris* GABA receptor and to displace $GABA_A$-receptor binding in mammalian brain is illustrated in Figure 9. Although we are comparing a system that measures affinity and efficacy with one that indicates the affinity of compounds only (binding) it can be seen that the relative order of potency of the compounds is roughly comparable in the two systems. Indeed, if we are justified in omitting the sulphonic acid derivatives from the calculations, on the basis of the evidence outlined above, then the two systems compare well with a significant ($P < 0.01$, positive ($r = 0.74$) correlation.

We conclude, therefore, that the GABA receptor on the somatic muscle cells of the parasitic nematode *Ascaris* is very similar to the mammalian $GABA_A$-receptor with regard to its activation by agonists. This would imply that the essential features of the receptor required for agonist recognition have been conserved through the course of evolution. The receptor does not recognise the typical $GABA_A$-competitive antagonists, however, indicating

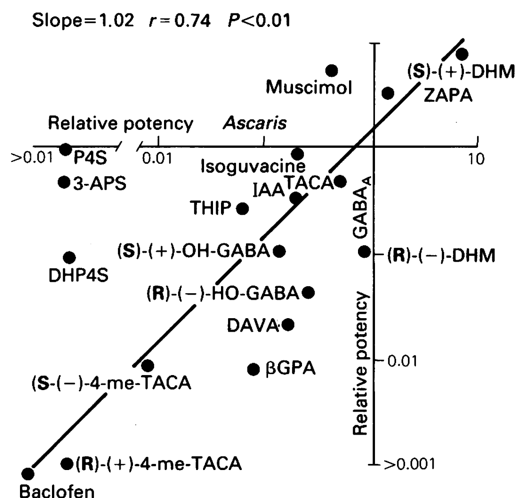

Figure 9 Correlation between the potency of compounds at displacing $GABA_A$-receptor binding (Krogsgaard-Larsen, 1988) or at GABA-mimetic activity in guinea-pig ileum [DAVA (Dickenson *et al.*, 1988), ZAPA (Allan *et al.*, 1986)] with their potency on *Ascaris* muscle cells (Holden-Dye & Walker, 1988; Holden-Dye *et al.*, 1988). The potency was determined as the relative potency compared to GABA in each system. The sulphonic acid derivatives were excluded from the calculation of the correlation coefficient; see text for details. (DHP4S, dihydropiperidine-sulphonic acid; 3-APS, 3-aminopropanesulphonic acid; IAA, imidazole acetic acid; DAVA, δ-aminovaleric acid; βGPA, β-guanidopropionic acid; ZAPA, (Z)-3-[amino-iminomethyl)thio]-2-propenoic acid hydrochloride).

that sites for antagonist binding at the receptor are different from those in the mammalian system. The chloride ionophore that mediates the GABA response in *Ascaris* must also be substantially different from the $GABA_A$-gated chloride channel in that none of the non-competitive antagonists for $GABA_A$-receptors was effective in *Ascaris*. Neither does the *Ascaris* GABA receptor exhibit the regulatory binding sites that are generally associated with the $GABA_A$-receptor. A similar receptor for GABA has recently been described in *Limulus* heart (Benson, 1988b). We have recently discovered that a diphenylamine-2-carboxylate derivative, 5-nitro-2-(3-phenylpropylamino)benzoic acid, which is known to block chloride transport in renal tubules (Wangemann *et al.*, 1986) is a noncompetitive antagonist at *Ascaris* GABA receptors (Colquhoun *et al.*, 1989). The possibility that there is a class of GABA receptor common to some invertebrate species with novel sites for antagonist recognition has important implications for pesticide and anthelmintic research.

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