Br. J. Pharmacol. (1988), **95**, 3–5

ZAPA, (Z)-3-[(aminoiminomethyl)thio]-2-propenoic acid hydrochloride, a potent agonist at GABA-receptors on the *Ascaris* muscle cell

L. Holden-Dye & R.J. Walker

Department of Neurophysiology, Medical & Biological Sciences Bldg., Bassett Crescent East, University of Southampton, Southampton SO9 3TU

This study is the first report of a compound which is equal in efficacy to γ-aminobutyric acid (GABA) at the nematode *Ascaris* muscle GABA-receptor. The GABA-receptor at the *Ascaris* muscle cell which mediates a membrane hyperpolarization and muscle relaxation has eluded classification. The structure-activity profile of this receptor is not typical of $GABA_A$ or $GABA_B$-receptors. Here we report that the isothiouronium compound ZAPA is as potent as GABA at this receptor. This finding has important implications for the characterization of the *Ascaris* GABA-receptor and the design of novel anthelmintics.

Introduction The *Ascaris* muscle γ-aminobutyric acid (GABA)-receptor-chloride ionophore complex is a promising target for anthelmintics. Two known anthelmintics, piperazine (del Castillo *et al.*, 1964; Martin, 1982) and Avermectin (Martin, 1987) have efficacy at this site and it is believed that this may underly their therapeutic action. However the pharmacological profile of this GABA-mediated response indicates that it does not readily fit into the vertebrate $GABA_A$ or $GABA_B$ classification (Hewitt *et al.*, 1986). No agonist has been found to be as potent as GABA. Muscimol, a GABA-mimetic with a higher potency than GABA in other invertebrate systems (Nistri & Constanti, 1979), only has a quarter of the efficacy of GABA in *Ascaris*. The anthelmintic piperazine has only one-tenth the potency of GABA. Both picrotoxin and bicuculline are inactive on this GABA-chloride ionophore complex (Wann, 1987).

The structure-activity relationships for the isothiouronium compounds have been described previously (Allan *et al.*, 1986). (Z)-3-[(aminoiminomethyl)thio]-2-propenoic acid hydrochloride (ZAPA) is described as the most potent of these compounds at GABA-receptors in assays such as contraction of the guinea-pig ileum and facilitation of [^{3}H]-diazepam binding. It has been described as a potent agonist at low affinity GABA-receptors associated with a benzodiazepine recognition site (Allan *et al.*, 1986). This study evaluated the efficacy of this compound at the *Ascaris* muscle GABA-receptor.

Methods Specimens of *Ascaris suum* were obtained from a local abattoir and maintained at 37°C in artificial perienteric fluid (APF, composition, mM: NaCl 67, Na acetate 67, KCl 3, $CaCl_2$ 3, $MgCl_2$ 15.7, glucose 3, Trizma base 5, pH 7.6). A 2 cm anterior section of the worm was taken and slit along one lateral line. This section was pinned cuticle side down on a Sylgard base in a perspex chamber and perfused with APF at 15 ml min^{-1}, 34°C. Intracellular recordings were made by impaling the bag region of the muscle cell with a 4 M K acetate filled electrode (10–30 MΩ). A second intracellular electrode was inserted to inject current pulses (20–35 nA, 500 mS pulse width, 0.1 Hz) and enable determination of membrane conductance. Membrane potentials and injected current pulses were monitored on a AXOCLAMP 2A and displayed on a GOULD 3000 2 channel pen recorder. Drugs made up in a volume of 10 ml were applied by perfusion over the preparation. Concentration-response curves for membrane hyperpolarization and conductance change were obtained for GABA and ZAPA in each cell studied. Only one cell was studied in each preparation.

In a few studies drugs were applied directly to the cell. This was achieved by use of a Picospritzer.

The EC_{50} value for each drug was determined by a direct computer-aided fit of the dose-response data using the least-squares curve-fitting programme of Barlow (1983). Hill plots of the data points between 10 and 90% of the maximum response were used to estimate the Hill coefficient of each drug. Differences between means were compared by the paired Student's *t* test.

GABA was supplied by BDH. ZAPA was supplied by Tocris Neuramin. ZAPA stock solutions were prepared immediately before use in HCl. The pH of stock dilutions was checked before use.

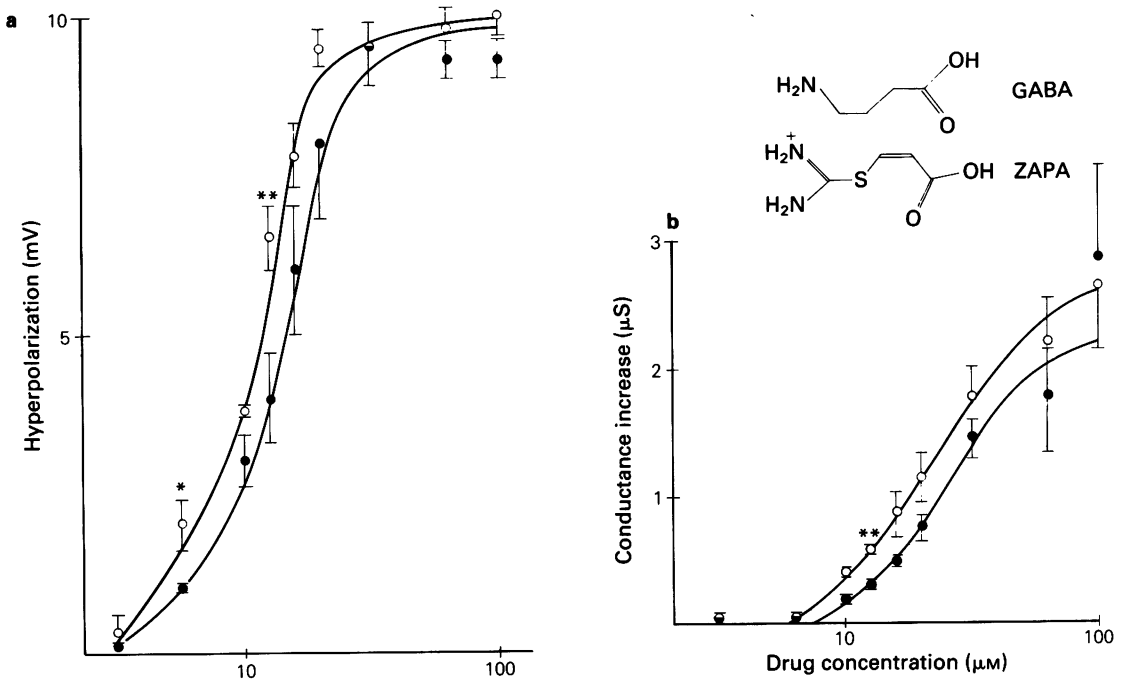

Figure 1 Concentration-response curves for GABA (●) and ZAPA (○) on (a) membrane hyperpolarization (b) increase in membrane conductance in *Ascaris* muscle cell. The cells were impaled with two microelectrodes, one for recording membrane potential and one for passing current pulses to enable determination of membrane conductance. Drugs were applied by perfusion over the preparation. A concentration-response curve was obtained for both GABA and ZAPA in each cell. Each point is the mean of 4 values with s.e.mean shown by vertical bars. $*P < 0.05$; $**P < 0.01$.

Results Muscle cell membrane potentials were in the range −30 to −40 mV. Typical resting membrane conductances were in the region of 2–2.5 μS. Figure 1 illustrates the comparative efficacy of GABA and ZAPA in inducing a membrane conductance increase and subsequent hyperpolarization on these cells. The EC_{50} for GABA on membrane hyperpolarization was 12.8 ± 0.4 μM compared to 10.3 ± 0.7 μM for ZAPA. Analysis of the conductance measurements indicated an EC_{50} of 42.3 ± 8.9 μM for GABA compared to 22.9 ± 1.2 μM for ZAPA ($n = 4$). The slope of the Hill plots derived from the conductance data for GABA and ZAPA were both greater than one, being 2.3 ± 0.3 and 2.4 ± 0.1 ($n = 4$, ±s.e.mean) respectively. Localized application of GABA or ZAPA (1 mM) onto the muscle cell by pressure ejection from a micropipette resulted in an identical response to that obtained by bath addition of the drugs. This supports the contention that the conductance change and resultant hyperpolarization initiated by bath addition of these drugs is mediated by a direct effect of the drug on a GABA-receptor on the muscle cell.

Discussion and conclusions The isothiouronium compound ZAPA is as potent as GABA in inducing membrane hyperpolarization of the *Ascaris* muscle cell. The EC_{50} for both GABA and ZAPA determined from conductance measurements are greater than those determined from membrane hyperpolarization and are probably a truer reflection of the efficacy of these compounds at the receptor. At two concentrations the response to ZAPA is significantly greater than that for GABA. There was, however, no significant difference between the EC_{50}s for GABA and ZAPA. The possibility that ZAPA is more potent than GABA in this system requires further investigation. Inactivation processes may be important in determining the relative efficacies of agents at a receptor. However, previous studies have shown that the GABA uptake blocker nipecotic acid does not potentiate the effects of GABA at the nematode somatic muscle GABA-receptor (Hewitt, 1987). Hill coefficients derived from the conductance data are near to 2 for both GABA and ZAPA. If it is assumed that each agonist receptor interaction results in a unit conductance change then this may be taken as

evidence that 2 molecules of GABA or ZAPA are required to activate each receptor. This concurs with observations made in other invertebrate systems (Nistri & Constanti, 1979) and points to some functional similarity between GABA-receptors in these systems and the nematode GABA-receptor.

Studies on vertebrate GABA-receptors have indicated that ZAPA interacts with a site coupled to a benzodiazepine recognition site (Allan *et al.*, 1986). Preliminary studies on the *Ascaris* GABA-receptor have failed to provide any evidence for such an association in this system (Hewitt, 1987). Further investigations will be carried out to clarify this point.

The results from this study indicate that the activity of the isothiouronium compounds on the nematode GABA receptor should be investigated both from the point of view of obtaining a clearer understanding of the structure-activity relationships at this receptor and with the aim of exploiting any possible anthelmintic potential of compounds such as ZAPA.

We wish to thank the SERC for financial support.

References

ALLAN, R.D., DICKENSON, H.W., HIERN, B.P., JOHNSTON, G.A.R. & KAZLAUSKAS, R. (1986). Isothiouronium compounds as γ-aminobutyric acid agonists. *Br. J. Pharmacol.*, **88**, 379–387.

BARLOW, R.B. (1983). *Biodata Handling with Microcomputers.* Amsterdam: Elsevier Biomedical Press.

DEL CASTILLO, J., DE MELLO, W.C. & MORALES, T.A. (1964). Inhibitory action of γ-aminobutyric acid (GABA) on *Ascaris* muscle. *Experientia*, **20**, 141–145.

HEWITT, G.M., WALKER, R.J. & WANN, K.T. (1986). Comparison of the effects of GABA receptor agonists on the somatic muscle receptors of *Ascaris suum. Br. J. Pharmacol.*, **89**, 789P.

HEWITT, G.M. (1987). Electrophysiological studies on the *Ascaris* muscle GABA receptor. *Ph.D. Thesis. Southampton University.*

MARTIN, R.J. (1982). Electrophysiological effects of piperazine and diethylcarbamazine on *Ascaris suum* somatic muscle. *Br. J. Pharmacol.*, **77**, 255–265.

MARTIN, R.J. (1987). γ-Aminobutyric acid receptors of *Ascaris* as a target for anthelmintics. *Biochem. Soc. Trans..*, **15**, 61–65.

NISTRI, A. & CONSTANTI, A. (1979). Pharmacological characterisation of different types of GABA and glutamate receptors in vertebrates and invertebrates. *Prog. Neurobiol.*, **13**, 117–235.

WANN, K.T. (1987). The electrophysiology of the somatic muscle cells of *Ascaris suum. Parasitology*, **94**, 555–566.

(Received May 3, 1988
Accepted May 13, 1988)