A NOVEL GABA RECEPTOR IN THE HEART OF A PRIMITIVE ARTHROPOD, *LIMULUS POLYPHEMUS*

By JACK A. BENSON

R & D Plant Protection, Agricultural Division, R-1093.P.47, CIBA-GEIGY Ltd, CH-4002 Basel, Switzerland

Summary

1. The isolated, intact heart of the marine arachnid *Limulus polyphemus* continues to beat *in vitro* for many hours. Application of γ-aminobutyric acid (GABA) decreased the heart beat frequency with a threshold of $3 \times 10^{-7}\,\text{mol}\,\text{l}^{-1}$ and an EC_{50} of $2.0 \pm 0.6 \times 10^{-5}\,\text{mol}\,\text{l}^{-1}$ (mean±S.D., $N = 8$). At $10^{-4}\,\text{mol}\,\text{l}^{-1}$ and above the heart beat was completely and reversibly inhibited.

2. The agonist potency profile of the *Limulus* heart chronotropic GABA receptor was very similar to that of the vertebrate $GABA_A$ receptor: muscimol > ZAPA>GABA≈TACA>isoguvacine>THIP>3-aminopropane sulphonic acid> imidazole-4-acetic acid ≈ β-guanidino proprionic acid ≈ 5-aminovalerate. In contrast, the antagonist profile differed dramatically: bicuculline, pitrazepin and SR 95103, as well as the channel blocker picrotoxin, were without effect at concentrations up to $10^{-4}\,\text{mol}\,\text{l}^{-1}$.

3. The benzodiazepines clorazepate, flunitrazepam, flurazepam and diazepam, as well as the barbiturate sodium pentobarbital, were without effect on the GABA response, suggesting that the *Limulus* heart GABA receptor is not complexed with the benzodiazepine and barbiturate modulatory subunits that characterize vertebrate $GABA_A$ receptor.

4. The $GABA_B$ ligands baclofen, phaclophen and kojic amine were inactive on the heart. However, 3-aminopropyl-phosphonous acid (CGA 147 823), a potent and highly selective $GABA_B$ agonist, was the most active of the compounds tested. It inhibited the heart beat with a threshold of about $3\,\text{nmol}\,\text{l}^{-1}$, an EC_{50} of $4.0 \pm 2.7 \times 10^{-7}\,\text{mol}\,\text{l}^{-1}$, and produced total inhibition of the heart at $10^{-5}\,\text{mol}\,\text{l}^{-1}$. CGA 147 823 was inactive on the locust thoracic somal GABA receptors.

5. *cis*-4-aminocrotonic acid (CACA), the ligand defining a proposed $GABA_C$-type receptor, was inactive on the heart.

6. The GABA-induced inhibition of the heart beat was enhanced by pretreatment with the GABA uptake inhibitor nipecotic acid but not with sodium valproate or β-alanine.

7. The *Limulus* heart chronotropic GABA receptor appears to be of a hitherto undescribed type that differs in pharmacology from the vertebrate $GABA_A$ and $GABA_B$ receptors as well as from the well-defined GABA receptors on the

somata of locust neurones and the muscle fibres of insects and the nematode *Ascaris*.

Introduction

Florey (1954) extracted a substance from the mammalian nervous system (Factor I) that had an inhibitory action on the crayfish stretch receptor. He showed that Factor I was largely γ-aminobutyric acid (GABA) (Bazemore *et al.* 1956) and that both Factor I and GABA slowed and stopped the heart beat of the crayfish *Astacus*, an effect antagonized by picrotoxin (Florey, 1957). At about the same time, Burgen and Kuffler (1957) demonstrated that GABA inhibited the endogenous rhythmic activity of the isolated cardiac ganglion from the neurogenic heart of a primitive marine arachnid *Limulus polyphemus*, the horseshoe crab. GABA is now recognised as the inhibitory neurotransmitter for the skeletal neuromuscular junction of crustaceans, insects and other arthropods, and it has inhibitory effects on some central neurones in these groups (Robinson and Olsen, 1988). GABA is equally important as an inhibitory neurotransmitter in vertebrate nervous systems where the GABA receptors so far described fall mostly into two categories, $GABA_A$ receptors and $GABA_B$ receptors (reviewed by Johnston, 1986). These receptor types are extremely well characterized pharmacologically and thus provide a starting point for the analysis of the pharmacology of GABA receptors in other phyla. Molecular genetic studies suggest that the $GABA_A$ and $GABA_B$ receptors are the products of different gene families, but classification of invertebrate GABA receptors still depends entirely on pharmacological criteria.

The heart of *Limulus* continues to beat for many hours when it is isolated intact and maintained in circulating physiological saline, and it responds chronotropically and inotropically to several biogenic amines and neuropeptides (Watson and Augustine, 1982). When the heart is exposed to GABA, the heart beat frequency and amplitude decrease (Pax and Sanborn, 1967; Abbott *et al.* 1969*a*). This report defines the pharmacology of the novel receptor type mediating the chronotropic effect of GABA on the heart beat. A preliminary note on this work has appeared previously (Benson, 1988*a*).

Materials and methods

Male and female specimens of *Limulus polyphemus*, with carapace widths of 15–25 cm, were obtained from the Department of Marine Resources at the Marine Biological Laboratory, Woods Hole, Massachussets and maintained in circulating artificial sea water (Instant Ocean) at 15°C.

The method for isolating the tubular heart and recording its contractions *in vitro* was as used previously (Benson *et al.* 1981). Part of the medial dorsal carapace was cut away. The heart was then removed by cutting the anterior and posterior blood vessels at their connection with the myocardium, leaving the cardiac ganglion intact on the dorsal surface of the heart. Any remaining connective tissue was

trimmed off the heart, which was then stretched by about 20 % of its length and pinned at both ends onto a wax surface in a plastic chamber (volume 30 ml). To measure contractions, a force transducer (Grass FT03) was connected by means of a hooked pin to the lateral boundary of the heart mid-way along its length. The heart was additionally pinned down at the lateral boundary opposite the transducer connection to improve the mechanics of the force transduction. The resulting contraction records were displayed on a polygraph recorder (Grass 79D). The preparation was continuously superfused at a rate of $15\,\mathrm{ml\,min^{-1}}$ with a physiological saline consisting of (in $\mathrm{mmol\,l^{-1}}$): NaCl, 445; $CaCl_2.6H_2O$, 10; $MgCl_2.6H_2O$, 46; KCl, 12; and Hepes, 5 (pH 7.4).

All test solutions were made up shortly before application. Poorly soluble test compounds were dissolved first in dimethylsulphoxide (DMSO) and then diluted with physiological saline. DMSO by itself was without effect on the heart beat at concentrations 10 times higher than those used here. The test compounds were purchased from Sigma except for the following: muscimol, isoguvacine, THIP, 3-aminopropane sulphonic acid, CGA 147 823, baclofen, kojic amine, phaclofen, flunitrazepam, sodium valproate (synthesized at CIBA-GEIGY Ltd), TACA, ZAPA, CACA (purchased from Tocris Neuramin), GABA, piperazine, piperidine-4-carboxylic acid (purchased from Fluka), SR 95103 (gift from Sanofi), pitrazepin (gift from Sandoz), clorazepate, flurazepam (gifts from Hoffmann-LaRoche) and sodium pentobarbital (purchased from Serva). The chemical names corresponding to abbreviations and code numbers are given in Table 1.

The experimental protocols were as follows. First, all the compounds were tested several times at $10^{-4}\,\mathrm{mol\,l^{-1}}$ for GABA-mimetic effects on the heart beat frequency. Those that were GABA agonists were further tested in an ascending series of concentrations, without intervening washes, to provide dose–response curves. Each concentration was perfused through the recording chamber for 10 min. The control frequency was taken from the 3 min preceding application of the first dose of the test compound, and the response was measured over the last 3 min of application of each concentration. For the conditions and compounds used in these experiments, the response measured was at a steady state. There was little evidence of desensitization of the GABAergic response. A similar lack of desensitization has been reported for the responses to the biogenic amines (Augustine *et al.* 1982).

With the exception of very potent agonists, all compounds were then tested for their effects on the magnitude of the chronotropic response to a standard GABA dose ($2\times10^{-5}\,\mathrm{mol\,l^{-1}}$, the EC_{50} for GABA), to detect antagonistic or modulatory influences. The standard GABA dose was applied 1–3 times for 10 min, with intervening 30-min intervals of perfusion with normal saline, to establish the magnitude of the control response. After 30 min of perfusion with normal saline, the test compound was applied for 30 min, followed without washout by the same concentration of test compound together with the standard GABA dose for 10 min. The mixture was then washed out and the preparation superfused with normal saline for 30 min, after which the standard GABA dose was tested again

(and repeated at 30-min intervals, if necessary) to determine the reversibility of any effects of the test compound, together with the stability of the GABA response. Although the magnitude of the GABA-induced frequency decrease varied somewhat from heart to heart, it was remarkably constant when the same dose of GABA was applied repeatedly to the same heart. This means that highly reliable control experiments could be carried out by using the protocol described above. The controls and response magnitudes in these experiments were measured as for the agonists.

Results

When the isolated heart was superfused with 2×10^{-5} mol l^{-1} GABA, the frequency of the heart beat decreased by about 50 % and there was also a small, transient decrease in the contraction amplitude (Fig. 1). The response was rapidly reversed by superfusion of the heart with control saline. It is not known whether different GABA receptor types mediate the chronotropic and inotropic components of the GABA response, but the present work is directed at the chronotropic response only. The dose–response curve shows that the threshold concentration was about 3×10^{-7} mol l^{-1} and the EC$_{50}$, calculated from individual dose–response curves, was $2.0\pm0.6\times10^{-5}$ mol l^{-1} ($\pm$s.d., $N=8$, Table 1A; Fig. 2). At 10^{-4} mol l^{-1} and above the heart beat was completely but reversibly inhibited.

The test compounds were grouped according to their known activities as agonists, antagonists and modulators at the vertebrate GABA$_A$, GABA$_B$ and

Control
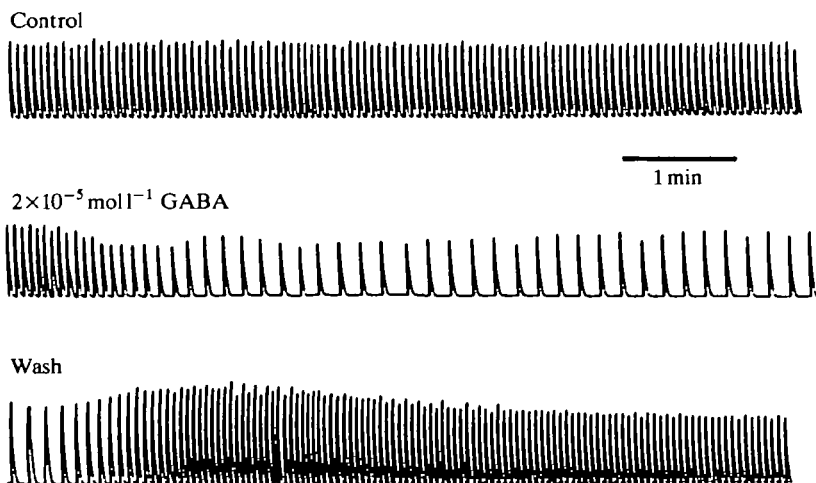

1 min

2×10^{-5} mol l^{-1} GABA

Wash

Fig. 1. Chart records of the GABA-induced decrease in heart beat frequency. The first trace shows the heart beat in control saline. The second trace illustrates the onset of the action of 2×10^{-5} mol l^{-1} GABA applied at the beginning of the trace. In the third trace, the reversal of the GABA inhibition by re-application of the control saline from the beginning of the trace is shown. The vertical axis is an arbitrary measurement of the heart beat amplitude.

Table 1. *GABAergic pharmacology of the* Limulus *heart*

	Agonism			Antagonism		
Compound	$EC_{50}\pm$s.D. (mol l^{-1})	Frequency change at 10^{-4} mol l^{-1}	No. of expts	% block of GABA response	Concentration tested (mol l^{-1})	No. of expts
A Putatively non-specific GABA agonists						
GABA	$2.0\pm0.6\times10^{-5}$	$-99\pm2\%$	8	–	–	0
Guanidino acetic acid	$4.5\pm0.9\times10^{-5}$	$-89\pm7\%$	3	NE	$10^{-5}, 3\times10^{-5}$	2
β-Guanidino proprionic acid		$-30\pm13\%$	3	NE	$10^{-5}, 10^{-4}$	3
Methapyrilene		$-11\pm7\%$	5	NE	10^{-4}	1
Piperazine		NE	7	NE	10^{-4}	4
Norvaline		NE	2	NE	10^{-4}	1
B Putatively non-specific GABA blockers						
Brucine		NE	3	$23\pm16\%$	10^{-4}	3
1-(*m*-chlorophenyl)-piperazine		Excitatory	3	NE	10^{-4}	2
Emetine		Excitatory	3	NE	10^{-4}	2
C Specific GABA$_A$ blockers						
Picrotoxin		NE	15	NE	10^{-4}	11
Bicuculline methiodide		NE	3	NE	10^{-4}	3
Pitrazepin		NE (10^{-5})	3	NE	10^{-5}	3
SR 95103		NE	3	NE	10^{-4}	3
D Specific GABA$_A$ agonists						
Muscimol	$4.3\pm1.3\times10^{-6}$	$-100\pm0\%$ (10^{-5})	3	–	–	0
ZAPA	$1.4\pm0.1\times10^{-5}$	$-100\pm0\%$	3	–	–	0
TACA	$4.0\pm0.9\times10^{-5}$	$-97\pm2\%$	3	–	–	0
Isoguvacine	$5.8\pm2.4\times10^{-5}$	$-77\pm14\%$	3	NE	3×10^{-6}	1
THIP		$-58\pm20\%$	5	NE	10^{-5}	1
3-Aminopropane sulphonic acid		$-32\pm12\%$	4	NE	10^{-4}	1
Imidazole-4-acetic acid		$-21\pm9\%$	4	NE	10^{-4}	2
5-Aminovalerate		$-16\pm9\%$	6	NE	10^{-4}	3
Isonipecotic acid		NE	3	NE	10^{-4}	1

Table 1. Continued

Compound	Agonism			Antagonism		
	EC$_{50}$±s.d. (mol l^{-1})	Frequency change at 10^{-4} mol l^{-1}	No. of expts	% block of GABA response	Concentration tested (mol l^{-1})	No. of expts
E Specific GABA$_B$ blocker						
Phaclofen		NE	6	NE	10^{-3}	3
F Specific GABA$_B$ agonists						
CGA 147 823	4.0±2.7×10^{-7}	−100±0% (10^{-5})	3	NE	10^{-6}	2
Baclofen		NE	4	NE	10^{-4}	2
Kojic amine		NE	5	NE	10^{-4}	1
G GABA$_C$ compound						
CACA		−7 and −20% (10^{-5})	2	NE	10^{-5}, 10^{-4}	2
		−5% (10^{-5})	1			
H Modulators						
Clorazepate		NE	12	NE	10^{-5}, 3×10^{-5}	11
Flunitrazepam		NE	5	NE	10^{-6}–3×10^{-5}	8
Flurazepam		NE	3	NE	10^{-5}, 10^{-4}	4
Diazepam		NE	2	NE	10^{-5}	2
Sodium pentobarbital		NE	5	NE	10^{-6}–10^{-4}	5
I GABA uptake and breakdown inhibitors						
Nipecotic acid		NE	7	+55±2%	10^{-4}	3
Sodium valproate		NE	2	NE	10^{-4}	2
β-Alanine		NE	3	NE	10^{-4}	3

Table 1. *Continued*

J Other compounds			Excitatory (10^{-5}, 10^{-4})		
Glycine	NE	3	NE	10^{-4}	1
Strychnine	NE	4	NE	10^{-4}	4
Norleucine	NE	1	NE	10^{-4}	1
Taurine	NE	5	NE	10^{-4}	3
Lindane	10^{-4}	5	NE	10^{-6}–10^{-4}	6

NE=no effect.
EC_{50}=median effective concentration.
Norvaline=L-2-aminopentanoic acid, L-α-aminovaleric acid.
Brucine=10,11-dimethoxystrychnine.
SR 95103=2-(carboxy-3'-propyl)-3-amino-4-methyl-6-phenylpyridazinium chloride.
ZAPA=(Z)-3-[(aminoiminomethyl)-thio]-prop-2-enoic acid.
TACA=*trans*-4-aminocrotonic acid.
THIP=4,5,6,7-tetrahydroisooxazolo-[5,4-c]-pyridin-3-ol.
5-Aminovalerate=5-aminopentanoic acid.
Isonipecotic acid=piperidine-4-carboxylic acid.
Phaclofen=3-amino-2-(4-chlorophenyl)-propyl phosphonic acid.
CGA 147 823=3-aminopropyl-phosphonous acid.
Baclofen=Lioresal, b-(*p*-chlorophenyl)-GABA.
Kojic amine=2-aminomethyl-5-hydroxy-4H-pyran-4-one.
CACA=*cis*-4-aminocrotonic acid.
Clorazepate=dipotassium hydroxide 7-chloro-2,3-dihydro-2-oxo-5-phenyl-1H-1,4-benzodiazepin-3-carboxylate.
Flurazepam=7-chloro-1-[2-(diethylamino)ethyl]-5-(o-fluorophenyl)-1,3-dihydro-2H-1,4-benzodiazepin-2-one.
Diazepam=7-chloro-1,3-dihydro-1-methyl-5-phenyl-2H-1,4-benzodiazepin-2-one.
Norleucine=L-2-aminohexanoic acid, L-α-aminocaproic acid.
Lindane=γ-hexa-chloro-cyclohexane.

$GABA_C$ receptors, and ranked within these groups in descending order of their potency on the *Limulus* heart (Table 1). The confidence limits given in Table 1 represent the mean±standard deviation of the EC_{50} values from individual dose–response curves.

Several reportedly non-specific GABA agonists were tested to determine whether any of them was more potent than GABA itself (Table 1A). Only guanidino acetic acid had an effect on the heart with a potency comparable to that of GABA. β-Guanidino proprionic acid was weakly active at 10^{-4} mol l^{-1}, as was methapyrilene, whose precise mode of GABAergic action is not known. It could be a partial agonist or antagonist at the $GABA_A$ receptor (Dalkara *et al.* 1986). Piperazine, an important anthelmintic that activates GABA channels on *Ascaris* muscle with a potency about one-hundredth that of GABA (Martin, 1985), had no effect on the heart at 10^{-4} mol l^{-1}. Norvaline, which shows some GABAergic effect at 10^{-4} mol l^{-1} and above on insect muscle (Scott and Duce, 1987), was completely inactive on the heart at 10^{-4} mol l^{-1}.

Of the three GABA blockers with undetermined specificity (Table 1B), only brucine acted as an antagonist on the heart, reducing the GABA responses by an average of about 25 % when applied at 10^{-4} mol l^{-1}. Brucine completely blocks the effect of GABA on [^{35}S]TBPS-binding at the $GABA_A$ receptors in the rat hippocampus and 1-(*m*-chlorophenyl)-piperazine acts similarly but with lower potency (Dalkara *et al.* 1986). Emetine, which was excitatory on the heart, is a weaker, partial blocker in the same system.

The original defining property of the $GABA_A$ receptor is specific antagonism by bicuculline (Hill and Bowery, 1981). Pitrazepin is also a selective antagonist and is more potent than bicuculline (Gähwiler *et al.* 1984). Bicuculline and pitrazepin were without effect on the *Limulus* heart receptor either as antagonists or agonists (Table 1C). A similar lack of effect was observed for SR 95103, one of the few $GABA_A$-specific competitive antagonists to retain the flexibility of the GABA moiety (Chambon *et al.* 1985).

The $GABA_A$ receptor gates a Cl$^-$ channel that is blocked by picrotoxin. This compound had no effect on the response of the heart to GABA, even after prolonged exposure at 10^{-4} mol l^{-1}. The GABA response of locust thoracic neurone somata is totally blocked by picrotoxin at less than 10^{-6} mol l^{-1} (Lees *et al.* 1987).

Characteristic agonists at the $GABA_A$ receptor (Table 1D) are muscimol, TACA, isoguvacine, THIP, 3-aminopropane sulphonic acid, the heterocyclic GABA analogue imidazole-4-acetic acid, 5-aminovalerate and isonipecotic acid, all with potencies similar to or greater than that of GABA (Krogsgaard-Larsen *et al.* 1977; reviewed by Krogsgaard-Larsen *et al.* 1985). The dose–response curves for the negative chronotropic effects of the more active of these compounds are shown in Fig. 2 and the EC_{50} values and effects at 10^{-4} mol l^{-1} are given with confidence limits in Table 1D. Muscimol and isoguvacine were active as agonists on the heart, the former being more potent than GABA itself. TACA, structurally restricted analogue of GABA with an extended conformation, was
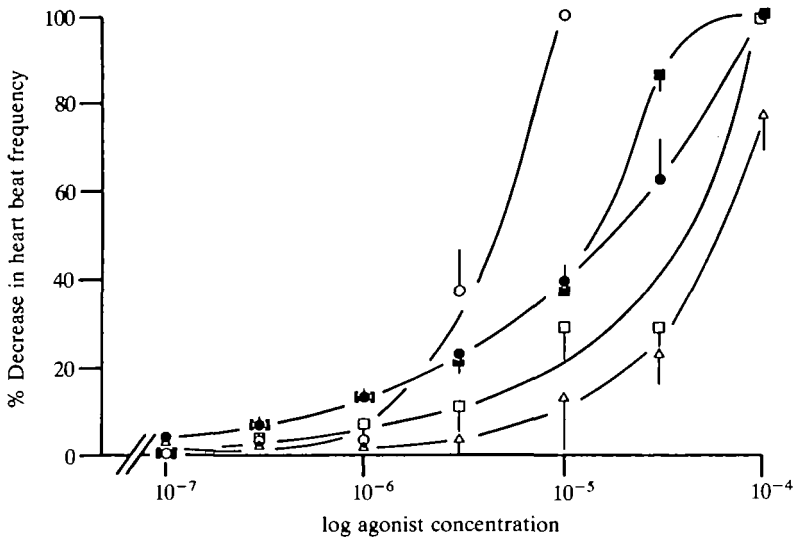
Fig. 2. Dose–response curves for the decrease in heart beat frequency induced by GABA and the active GABA$_A$ agonists. (●) GABA, $N=6$; (○) muscimol, $N=3$; (■) ZAPA, $N=3$; (□) TACA, $N=3$; (△) isoguvacine, $N=3$. The points indicate the means and the vertical bars represent the standard errors where these are large enough to extend beyond the points. The concentrations are measured in $mol\,l^{-1}$.

slightly less potent than GABA. A similar result has been reported for the insect muscle GABA receptor (Scott and Duce, 1987). However, THIP showed significant activity only at high concentrations. 3-Aminopropane sulphonic acid was only feebly active at $10^{-4}\,mol\,l^{-1}$ (as at many invertebrate GABA receptors, Simmonds, 1983), and imidazole-4-acetic acid and 5-aminovalerate were extremely weak but, nevertheless, active. Their rank order of potency on the heart was almost identical to that on the GABA$_A$ receptors of cultured mouse spinal neurones: muscimol > GABA $\approx$ TACA > isoguvacine $\approx$ THIP > 3-aminopropane sulphonic acid $\approx$ β-guanidino proprionic acid $\approx$ 5-aminovalerate > imidazole-4-acetic acid (Barker and Mathers, 1981). Isonipecotic acid, reputedly active selectively at GABA$_A$ receptors (Krogsgaard-Larsen *et al.* 1985), was inactive on the heart. ZAPA has become available relatively recently. It is more potent than muscimol at the low-affinity binding site of the GABA$_A$ receptor (Allan *et al.* 1986) and was more active than TACA but less active than muscimol on the heart.

The folded isomer of TACA, CACA, was inactive on the heart (Table 1G). CACA-binding is the defining characteristic of a proposed baclofen- and bicuculline-insensitive GABA$_C$ receptor (Drew *et al.* 1984; Johnston, 1986).

The GABA$_B$ receptor was originally defined as being specifically activated by baclofen and, in addition, insensitive to bicuculline (Bowery *et al.* 1980). It gates a K$^+$ channel (Gähwiler and Brown, 1985). Baclofen was without effect on the heart (Table 1F). Phaclofen, the phosphonic acid derivative of baclofen, is a low-potency, highly selective antagonist at the GABA$_B$ receptor (Kerr *et al.* 1987;
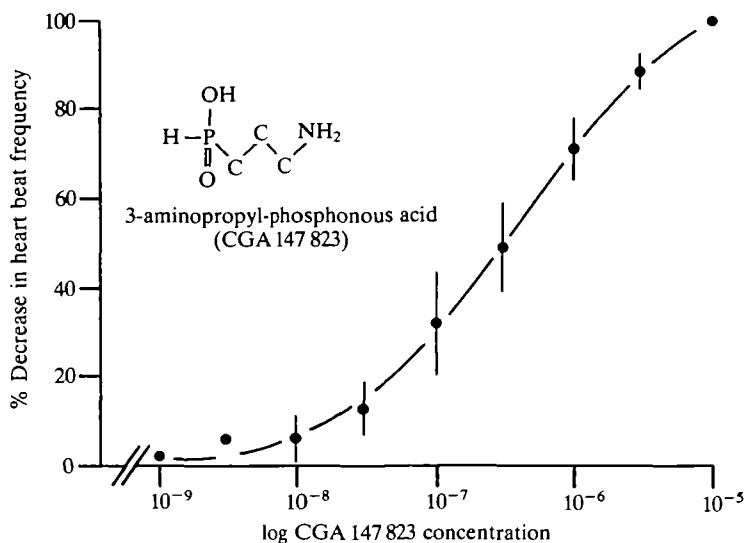
Fig. 3. Dose–response curve for the decrease in heart beat frequency induced by CGA 147 823 (3-aminopropyl-phosphonous acid). The points indicate the means and the vertical bars represent the standard errors where these are large enough to extend beyond the points ($N=3$, except for the lowest two concentrations which are single measurements). Concentrations are measured in $mol\,l^{-1}$.

Dutar and Nicoll, 1988). It also had no agonistic or antagonistic action on the heart at concentrations up to $10^{-3}\,mol\,l^{-1}$ (Table 1E). Kojic amine was designed as a $GABA_A$ agonist but in fact is primarily active as an agonist at $GABA_B$ receptors (Krogsgaard-Larsen *et al.* 1985). It had no effect either as an agonist or an antagonist on the heart (Table 1F).

Several γ-aminopropyl-phosphonous acids, which are GABA agonists in hippocampal slices, have been reported to bind at $GABA_B$ receptors potently and with high specificity (Dingwall *et al.* 1985, 1987). CGA 147 823 (3-aminopropyl-phosphonous acid) exhibits very highly selective binding to the vertebrate $GABA_B$ receptor ($GABA_B$ $IC_{50} = 1 \times 10^{-8}\,mol\,l^{-1}$, no effect on $GABA_A$ binding at $10^{-5}\,mol\,l^{-1}$; H. Bittiger, Pharmaceutical Division, CIBA-GEIGY Ltd, Basel, personal communication). When applied to the *Limulus* heart, this compound decreased the beat frequency with a potency 10-fold greater than that of muscimol (Table 1F), which makes it the most active of the compounds tested. As shown in Fig. 3 and Table 1F, it had an activity threshold of about $3\,nmol\,l^{-1}$, an EC_{50} of $4.0 \pm 2.7 \times 10^{-7}\,mol\,l^{-1}$ and produced total inhibition of the heart at $10^{-5}\,mol\,l^{-1}$.

The vertebrate $GABA_A$ receptor is complexed with receptors for benzodiazepines and barbiturates, compounds that enhance the action of GABA. The locust thoracic neurone somal GABA receptor also forms part of such a complex, and flunitrazepam, for example, increases the GABA response by up to 70 % when applied for 10 min at $10^{-5}\,mol\,l^{-1}$ and sodium pentobarbital is active $5 \times 10^{-5}\,mol\,l^{-1}$ (Lees *et al.* 1987). Four benzodiazepines and one barbiturate we

Control

$2 \times 10^{-5}\,\text{mol}\,\text{l}^{-1}$ GABA

Wash

$10^{-4}\,\text{mol}\,\text{l}^{-1}$ nipecotic acid

$10^{-4}\,\text{mol}\,\text{l}^{-1}$ nipecotic acid $+ 2 \times 10^{-5}\,\text{mol}\,\text{l}^{-1}$ GABA

Wash

$2 \times 10^{-5}\,\text{mol}\,\text{l}^{-1}$ GABA
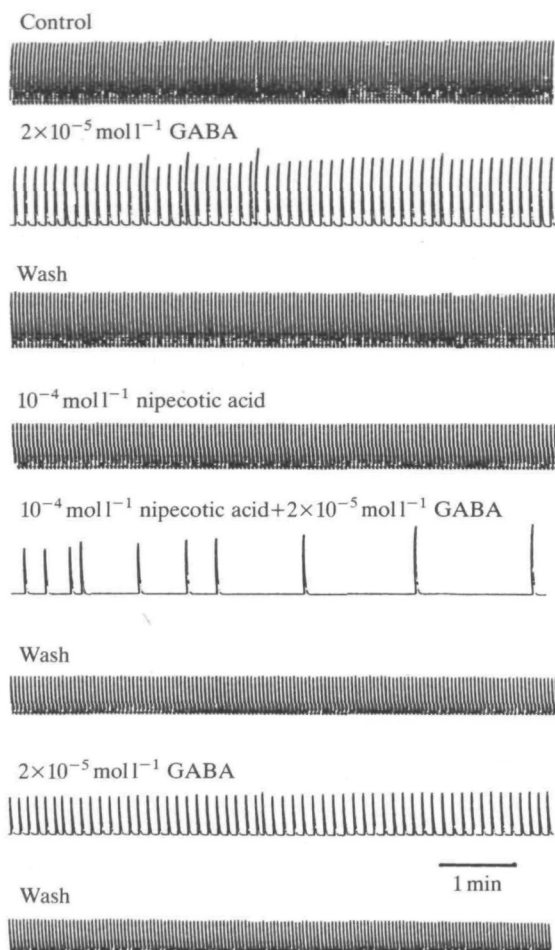
1 min

Wash

Fig. 4. Chart records showing the enhancement of the GABA-induced decrease in heart beat frequency by nipecotic acid. 'Wash' traces illustrate the steady-state heart beat following re-application of the control saline. The vertical axis is an arbitrary measurement of the heart beat amplitude.

tested on the heart for modulatory effects on the GABA response (Table 1H). They were all without effect: the GABA responses in the presence of the putative modulators were not significantly different from those in control saline.

Nipecotic acid, a specific inhibitor of GABA uptake (Krogsgaard-Larsen and Johnston, 1975), had no agonistic effect in the absence of GABA but produced a dramatic enhancement of the GABA response of the heart (Table 1I and Fig. 4). In contrast, sodium valproate and β-alanine, which are also GABA uptake inhibitors in some systems, were ineffective on the heart. β-Alanine had no effect by itself on the heart, although it is reported to evoke a GABA-mimetic effect on the skeletal muscle of the locust at very high concentrations (Scott and Duce, 1987).

Table 1J presents the results of experiments testing the effects of a selection of compounds that might be expected to be active on a GABA receptor/chloride channel complex. Glycine is an important mammalian neurotransmitter that activates a receptor/chloride channel complex strongly homologous in primary structure to both the $GABA_A$ receptor and the nicotinic acetylcholine receptor (reviewed by Betz and Becker, 1988). Strychnine potently antagonizes the vertebrate glycine receptor (Betz and Becker, 1988) and the locust thoracic neurone somal nicotinic receptor (Benson, 1988c). Neither glycine nor strychnine was active on the heart GABA receptor, and nor were taurine and norleucine, possible GABA agonists. Lindane, an insecticide that blocks an insect neuronal GABA response at $10^{-4}\,\mathrm{mol\,l^{-1}}$ (Wafford et al. 1988), probably by acting on the Cl^- channel, did not block the GABA response in the heart, but had an excitatory effect at 10^{-5} and $10^{-4}\,\mathrm{mol\,l^{-1}}$.

Discussion

GABA receptors can be classified into two families: $GABA_A$, members of the ligand-binding-activated superfamily, and $GABA_B$, members of the G-protein-associated superfamily (reviewed by Johnston, 1986). The *Limulus* heart receptor has an agonist profile (Table 1D) very similar to at least one well-characterized vertebrate $GABA_A$ receptor (Barker and Mathers, 1981), but it has a different GABA recognition site antagonist profile (Table 1C), being insensitive to bicuculline, the diagnostic $GABA_A$ antagonist, and pitrazepin, as well as to SR 95103. Similar observations have been reported for other arthropods. Insect neuronal GABA receptors, especially those on the soma, are generally not blocked by bicuculline (reviewed with the exceptions by Benson, 1988b) or pitrazepin (Lees et al. 1987), and insensitivity to bicuculline has been observed for crustacean muscular GABA responses (e.g. Takeuchi and Onodera, 1972) and binding sites (e.g. Fiszer de Plazas and De Robertis, 1973) and *Limulus* CNS neurones (Walker and Roberts, 1982). The neuronal and muscular GABA receptors of insects probably belong to the same family as the vertebrate $GABA_A$ receptor complex (Benson, 1988c). This hypothesis is supported by data derived from both binding studies (Lunt et al. 1985; Lummis and Sattelle, 1985a; Breer and Heilgenberg, 1985) and electrophysiology (Neumann et al. 1987). However, like the vertebrate $GABA_A$ receptor, the insect GABA receptors so far described gate a Cl^- channel that is blocked by picrotoxin (e.g. Lees et al. 1987). In this respect, the *Limulus* heart GABA receptor is markedly different, being totally insensitive to picrotoxin (Table 1C; Pax and Sanborn, 1967), and it is thus different from all other receptors so far classed in the $GABA_A$ receptor family. It is noteworthy that neuronal GABA responses in the autonomously rhythmic stomatogastric ganglia in crustaceans, which are functionally and probably evolutionarily homologous to the cardiac ganglion, are also unusually resistant to blockade by picrotoxin (Marder and Paupardin-Tritsch, 1978; Albert et al. 1986; Cazelets et al. 1987). More extensive pharmacological profiles for the picrotoxin-insensitive GABA

receptors in lobsters and crabs would be useful in determining whether they belong to the same subtype as the *Limulus* heart receptor. A picrotoxin-insensitive GABA response has been described in fish retinal horizontal cells but this response is blocked by bicuculline (Hankins and Ruddock, 1984).

The GABA receptors in the *Limulus* heart differ from those in the *Limulus* CNS, which appear to be similar in many respects to those on insect neurones and thus, probably, GABA$_A$ type receptors (James *et al.* 1978; Roberts *et al.* 1981; Walker *et al.* 1981; Walker and Roberts, 1982). Whereas the GABA response of the heart was unaffected by picrotoxin, GABA hyperpolarizes neurones in the CNS of *Limulus* by activating a Cl$^-$ conductance that is blocked by low concentrations of this compound. As on the heart, muscimol is more potent than GABA and isoguvacine is about equipotent. However, THIP and piperidine-4-carboxylic acid (isonipecotic acid) are approximately equipotent with GABA in the CNS but were much weaker (THIP) or inactive (isonipecotic acid) on the heart. Bicuculline and baclofen were inactive on both the CNS and heart.

Another important characteristic of the vertebrate GABA$_A$ receptor is that it is associated with benzodiazepine and barbiturate regulatory sites that are absent from GABA$_B$ receptors. The *Limulus* heart receptor appears to have no benzodiazepine or barbiturate allosteric regulatory sites associated with it (Table 1H). The presence of benzodiazepine binding sites in arthropods was first demonstrated in housefly thorax muscles (Abalis *et al.* 1983). Similar binding sites were later identified on neuronal membranes from insects (Robinson *et al.* 1985; Lummis and Sattelle, 1985*b*). At the same time, it was shown electrophysiologically that locust thoracic neurone somata possess functional benzodiazepine receptors and that functional barbiturate receptors also modulate the GABA responses of these insect neurones (Lees *et al.* 1985, 1987). Scott and Duce (1987) observed barbiturate potentiation of the insect muscular GABA response and Shimahara *et al.* (1987) demonstrated potentiation by a benzodiazepine of GABA single-channel activation in cultured insect neurones. This is additional evidence that these insect GABA receptors belong to the GABA$_A$ superfamily. Robinson *et al.* (1986) found insect benzodiazepine binding sites that differ in pharmacological detail from that of the vertebrate GABA$_A$ receptor. It is therefore conceivable that the *Limulus* receptor is complexed with allosteric sites that are insensitive to the compounds tested in the experiments reported here. Nevertheless, the most plausible conclusion from the data is that the *Limulus* heart receptor is not closely related to the other members of the GABA$_A$ family.

The *Limulus* heart receptor is insensitive to the GABA$_B$ diagnostic agonist, baclofen, and is not blocked by phaclofen, a weak but highly specific GABA$_B$ antagonist. The pharmacology of the *Limulus* receptor GABA recognition site thus clearly differs from that of the vertebrate GABA$_B$ receptor. However, CGA 147 823 was 10 times more potent than muscimol on the heart, and in the vertebrate brain it is a highly specific GABA$_B$ agonist. This compound has no agonistic or antagonistic GABAergic effects at concentrations of up to 10^{-4} mol l^{-1} on locust thoracic neuronal somata that responded with high

sensitivity to GABA (J. A. Benson, unpublished data). This is perhaps the most striking difference between the GABA recognition site pharmacological profiles of the *Limulus* heart and the locust CNS, and illustrates the remarkable diversity to be found among receptors for the same transmitter within a single phylum. A definitive answer on whether the *Limulus* receptor could belong to the same superfamily as the $GABA_B$ receptor awaits biochemical clarification of the rôle, if any, of cyclic GMP as a second messenger in the *Limulus* GABA response, or determination of the amino acid sequence of the receptor protein. On the basis of purely pharmacological data, however, the *Limulus* receptor is clearly not closely related to the vertebrate $GABA_B$ receptor.

A GABA receptor that does not fit the $GABA_A$/$GABA_B$ classification is the GABA-activated Cl^--ionophore that occurs in *Ascaris* muscle cells. On this preparation, the rank order of agonist potency is ZAPA > GABA > TACA > muscimol > imidazole-4-acetic acid ≈ isoguvacine > guanidino acetic acid > 3-guanidinoproprionic acid ≈ 5-aminovalerate > CACA > THIP (Holden-Dye *et al.* 1988). Although showing similarities in the relative potencies for several of these compounds, the *Ascaris* muscle receptor profile differs from that of the *Limulus* heart with respect to the low potencies of muscimol (about 25 % that of GABA) and THIP (about 0.5 % that of GABA). ZAPA is the only compound reported so far to have a potency at the *Ascaris* receptor equal to or slightly greater than that of GABA (Holden-Dye and Walker, 1988), and in this respect it resembles the heart. Also, as in the heart, picrotoxin, pitrazepin and bicuculline are inactive (Wann, 1987), as are baclofen, glycine, β-alanine and taurine (Holden-Dye *et al.* 1988). In contrast, 3-aminopropane sulphonic acid (10^{-3} mol l^{-1}) is inactive on the *Ascaris* muscle receptor but showed agonistic activity at 10^{-4} mol l^{-1} on the heart (Table 1D). High doses of piperazine are reported to activate single GABA channels on *Ascaris* muscle (Martin, 1985) but this compound is inactive on the heart (Table 1A). There is clearly an overall similarity in several critical respects between the *Ascaris* muscle and the *Limulus* heart receptors: the differences appear to be real but occur in the details of the relative potencies of the agonists.

Simmonds (1983) hypothesized that invertebrates possess only 'simple' GABA receptors that do not form part of a complex including allosteric regulatory sites. This is not the case in the insects, but the *Limulus* heart chronotropic GABA receptor does seem to fit this concept well. In addition to lacking the regulatory sites, the *Limulus* receptor has a pharmacological profile that differs markedly from those of both $GABA_A$ and $GABA_B$ vertebrate receptors, and it is insensitive to the diagnostic agonist of the proposed $GABA_C$ receptor. It does not match the pharmacology of the GABA receptors of *Limulus* central neurones, locust thoracic neurones, insect muscle or the GABA receptor on the muscle fibres of the nematode *Ascaris*.

GABA uptake appears to be important in the heart. Nipecotic acid enhanced the GABA response and, since it is a specific inhibitor of GABA uptake in the vertebrate brain (Krogsgaard-Larsen *et al.* 1985) and blocks GABA uptake in cultured insect neurones (Bermudez *et al.* 1988), this result suggests that it might

be acting similarly in the *Limulus* heart, effectively increasing the amount of GABA available by reducing its uptake. β-Alanine, an inhibitor of GABA uptake into glia in many preparations, including the insect nervous system (Beadle *et al.* 1987), was without effect. GABA uptake is therefore probably a neuronal phenomenon in the heart.

The precise rôle in physiological cardioinhibition played by the GABA receptor characterized here is not settled. Pax and Sanborn (1967) discounted the possibility that GABA could be the transmitter released by the inhibitory cardioregulatory nerves because stimulation of the nerves had both negative chronotropic and inotropic effects, whereas they thought that GABA affected primarily the frequency. The experiments described here, and previous data (Abbott *et al.* 1969a,b), show that GABA reduces both the amplitude and the frequency of the heart beat. These effects, which mimic stimulation of the inhibitory cardioregulatory nerves in every way, appear to be mediated solely by the cardiac ganglion, where GABA causes a reduction in the frequency and duration of the bursts recorded as well as in the number of cardiac ganglion units firing during a burst (Abbott *et al.* 1969a). GABA has no effect on the neuromuscular EPSP or heart muscle contractility (Abbott *et al.* 1969b). These observations strongly suggest that the inhibitory cardioregulatory nerves release GABA in the cardiac ganglion and that the changes in burst characteristics account for both the chronotropic and the inotropic effects on the heart beat. Unfortunately, Pax and Sanborn (1967) also showed that picrotoxin blocks the effects of stimulation of the inhibitory cardioregulatory nerves but, as the above results confirm, does not block the action of GABA. This means that if GABA has a physiological rôle in the *Limulus* heart, it is more likely to be as a blood-borne neuromodulator acting on the cardiac ganglion neurones than as a cardiac inhibitory neurotransmitter.

References

ABALIS, I. M., ELDEFRAWI, M. E. AND ELDEFRAWI, A. T. (1983). Biochemical identification of putative GABA/benzodiazepine receptors in house fly thorax muscles. *Pesticide Biochem. Physiol.* **20**, 39–48.

ABBOTT, B. C., LANG, F. AND PARNAS, I. (1969a). Physiological properties of the heart and cardiac ganglion of *Limulus polyphemus*. *Comp. Biochem. Physiol.* **28**, 149–158.

ABBOTT, B. C., LANG, F., PARNAS, I., PARMLEY, W. AND SONNENBLICK, E. (1969b). Physiological and pharmacological properties of *Limulus* heart. *Experientia (Suppl.)* **15**, 232–243.

ALBERT, J., LINGLE, C. J., MARDER, E. AND O'NEIL, M. B. (1986). A GABA-activated chloride-conductance not blocked by picrotoxin on spiny lobster neuromuscular preparations. *Br. J. Pharmac.* **87**, 771–779.

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

AUGUSTINE, G. J., FETTERER, R. AND WATSON, W. H. (1982). Amine modulation of the neurogenic *Limulus* heart, *J. Neurobiol.* **13**, 61–74.

BARKER, J. L. AND MATHERS, D. A. (1981). GABA analogues activate channels of different duration on cultured mouse spinal neurons. *Science* **212**, 358–361.

BAZEMORE, A., ELLIOTT, K. A. C. & FLOREY, E. (1956). Factor I and γ-aminobutyric acid. *Nature, Lond.* **178**, 1052–1053.

BEADLE, C. A., BERMUDEZ, I. & BEADLE, D. J. (1987). Amino acid uptake by neurones and glial cells from embryonic cockroach brain growing *in vitro*. *J. Insect Physiol.* **33**, 761–768.

BENSON, J. A. (1988a). The GABA response of the isolated heart of *Limulus polyphemus* exhibits novel pharmacology. *Soc. Neurosci. Abstr.* **14**, 382.

BENSON, J. A. (1988b). Bicuculline blocks the response to acetylcholine and nicotine but not to muscarine or GABA in isolated insect neuronal somata. *Brain Res.* **458**, 65–71.

BENSON, J. A. (1988c). Transmitter receptors on insect neuronal somata: GABAergic and cholinergic pharmacology. In *The Molecular Basis of Drug and Pesticide Action – Neurotox '88* (ed. G. G. Lunt), pp. 193–206. Amsterdam: Elsevier Biomedical.

BENSON, J. A., SULLIVAN, R. E., WATSON, W. H. AND AUGUSTINE, G. J. (1981). The neuropeptide proctolin acts directly on *Limulus* cardiac muscle to increase the amplitude of contraction. *Brain Res.* **213**, 449–454.

BERMUDEZ, I., BOTHAM, R. P. AND BEADLE, D. J. (1988). High- and low-affinity uptake of amino acid transmitters in cultured neurones and muscle cells of the cockroach, *Periplaneta americana*. *Insect Biochem.* **18**, 249–262.

BETZ, H. & BECKER, C.-M. (1988). The mammalian glycine receptor: biology and structure of a neuronal chloride channel protein. *Neurochem. Int.* **13**, 137–146.

BOWERY, N. G., HILL, D. R., HUDSON, A. L., DOBLE, A., MIDDLEMISS, D. N., SHAW, J. & TURNBULL, M. (1980). (−)Baclofen decreases neurotransmitter release in the mammalian CNS by an action at a novel GABA receptor. *Nature, Lond.* **283**, 92–94.

BREER, H. AND HEILGENBERG, H. (1985). Neurochemistry of GABAergic activities in the central nervous system of *Locusta migratoria*. *J. comp. Physiol.* A **157**, 343–354.

BURGEN, A. S. V. AND KUFFLER, S. W. (1957). The inhibition of the cardiac ganglion of *Limulus polyphemus* by 5-hydroxytryptamine. *Biol. Bull. mar. biol. Lab., Woods Hole* **113**, 336.

CAZALETS, J. R., COURNIL, I., GEFFARD, M. AND MOULINS, M. (1987). Suppression of oscillatory activity in crustacean pyloric neurons: implication of GABAergic inputs. *J. Neurosci.* **7**, 2884–2893.

CHAMBON, J.-P., FELTZ, P., HEAULME, M., RESTLE, S., SCHLICHTER, R., BIZIERE, K. AND WERMUTH, C. G. (1985). An arylaminopyridazine derivative of γ-aminobutyric acid (GABA) is a selective and competitive antagonist at the GABA$_A$ receptor site. *Proc. natn. Acad. Sci. U.S.A.* **82**, 1832–1836.

DALKARA, T., SAEDERUP, E., SQUIRES, R. F. AND KRNJEVIC, K. (1986). Iontophoretic studies on rat hippocampus with some novel GABA antagonists. *Life Sci.* **39**, 415–422.

DINGWALL, J. G., EHRENFREUND, J., HALL, R. G. AND JACK, J. (1985). Substituted propane-phosphonous acid compounds. *European Patent Application. Publication number* 0 181 833.

DINGWALL, J. G., EHRENFREUND, J., HALL, R. G. AND JACK, J. (1987). Synthesis of γ-aminopropylphosphonous acids using hypophosphorous acid synthons. *Phosphorus and Sulfur* **30**, 571–574.

DREW, C. A., JOHNSTON, G. A. R. AND WEATHERBY, R. P. (1984). Bicuculline-insensitive GABA receptors: studies on the binding of (−)baclofen to rat cerebellar membranes. *Neurosci. Letters* **52**, 317–321.

DUTAR, P. & NICOLL, R. A. (1988). A physiological role for GABA$_B$ receptors in the central nervous system. *Nature, Lond.* **332**, 156–158.

FISZER DE PLAZAS, S. & DE ROBERTIS, E. (1973). Hydrophobic proteins isolated from crustacean muscle having glutamate and γ-aminobutyrate receptor properties. *FEBS Lett.* **33**, 45–48.

FLOREY, E. (1954). An inhibitory and an excitatory factor of mammalian central nervous system, and their action on a single sensory neuron. *Arch. Int. Physiol.* **62**, 33–53.

FLOREY, E. (1957). Further evidence for the transmitter-function of Factor I. *Naturwissenschaften* **44**, 424–425.

GÄHWILER, B. H. AND BROWN, D. A. (1985). GABA$_B$-receptor-activated K$^+$ current in voltage-

clamped CA$_3$ pyramidal cells in hippocampal cultures. *Proc. natn. Acad. Sci. U.S.A.* **82**, 1558–1562.

GÄHWILER, B. H., MAURER, R. & WÜTHRICH, H. J. (1984). Pitrazepin, a novel GABA$_A$ antagonist. *Neurosci. Lett.* **45**, 311–316.

HANKINS, M. W. & RUDDOCK, K. H. (1984). Electrophysiological effects of GABA on fish retinal horizontal cells are blocked by bicuculline but not by picrotoxin. *Neurosci. Lett.* **44**, 1–6.

HILL, D. R. AND BOWERY, N. G. (1981). ^{3}H-Baclofen and ^{3}H-GABA bind to bicuculline-insensitive GABA$_B$ sites in rat brain. *Nature, Lond.* **290**, 149–152.

HOLDEN-DYE, L., HEWITT, G. M., WANN, K. T., KROGSGAARD-LARSEN, P. AND WALKER, R. J. (1988). Studies involving avermectin and 4-aminobutyric acid (GABA) receptor of *Ascaris suum* muscle. *Pestic. Sci.* **24**, 231–245.

HOLDEN-DYE, L. AND WALKER, R. J. (1988). ZAPA, (Z)-3-[(aminoiminomethyl)thio]-2-propenoic acid hydrochloride, a potent agonist at the GABA-receptors on the *Ascaris* muscle cell. *Br. J. Pharmac.* **95**, 3–5.

JAMES, V. A., KROGSGAARD-LARSEN, P. & WALKER, R. J. (1978). The action of conformationally restricted analogues of GABA on *Limulus* and *Helix* central neurones. *Experientia* **34**, 1630–1631.

JOHNSTON, G. A. R. (1986). Multiplicity of GABA receptors. In *Benzodiazepine/GABA Receptors and Chloride Channels: Structural and Functional Properties* (ed. R. W. Olsen & J. C. Venter), pp. 57–71. New York: Alan R. Liss.

KERR, D. I. B., ONG, J., PRAGER, R. H., GYNTHER, B. D. AND CURTIS, D. R. (1987). Phaclofen: a peripheral and central baclofen antagonist. *Brain Res.* **405**, 150–154.

KROGSGAARD-LARSEN, P., FALCH, E. AND HJEDS, H. (1985). Heterocyclic analogues of GABA: chemistry, molecular pharmacology and therapeutic aspects. *Prog. Med. Chem.* **22**, 67–119.

KROGSGAARD-LARSEN, P. AND JOHNSTON, G. A. R. (1975). Inhibition of GABA uptake in rat brain slices by nipecotic acid, various isoxazoles and related compounds. *J. Neurochem.* **25**, 797–802.

KROGSGAARD-LARSEN, P., JOHNSTON, G. A. R., LODGE, D. AND CURTIS, D. R. (1977). A new class of GABA agonist. *Nature, Lond.* **268**, 53–55.

LEES, G., BEADLE, D. J., NEUMANN, R. & BENSON, J. A. (1987). Responses to GABA by isolated insect neuronal somata: pharmacology and modulation by a benzodiazepine and a barbiturate. *Brain Res.* **401**, 267–278.

LEES, G., NEUMANN, R., BEADLE, D. J. AND BENSON, J. A. (1985). Flunitrazepam enhances responses induced by 4-aminobutyric acid and muscimol in freshly dissociated locust central neuronal somata. *Pesticide Sci.* **16**, 534.

LUMMIS, S. C. R. AND SATTELLE, D. B. (1985a). Insect central nervous system γ-aminobutyric acid. *Neurosci. Lett.* **60**, 13–18.

LUMMIS, S. C. R. AND SATTELLE, D. B. (1985b). Binding sites for 4-aminobutyric acid and benzodiazepines in the central nervous system of insects. *Pesticide Sci.* **16**, 695–697.

LUNT, G. G., ROBINSON, T. N., MILLER, T., KNOWLES, W. P. AND OLSEN, R. W. (1985). The identification of GABA receptor binding sites in insect ganglia. *Neurochem. Int.* **7**, 751–754.

MARDER, E. & PAUPARDIN-TRITSCH, D. (1978). The pharmacological properties of some crustacean neuronal acetylcholine, γ-aminobutyric acid, and L-glutamate responses. *J. Physiol., Lond.* **280**, 213–236.

MARTIN, R. J. (1985). γ-Aminobutyric acid- and piperazine-activated single-channel currents from *Ascaris suum* body muscle. *Br. J. Pharmac.* **84**, 445–461.

NEUMANN, R., LEES, G., BEADLE, D. J. AND BENSON, J. A. (1987). Responses to GABA and other neurotransmitters in insect central neuronal somata *in vitro*. In *Sites of Action for Neurotoxic Pesticides* (ed. R. M. Hollingworth & M. B. Green), pp. 25–43. Washington, DC: American Chemical Society.

PAX, R. A. AND SANBORN, R. C. (1967). Cardioregulation in *Limulus*. II. Gamma aminobutyric acid, antagonists and inhibitor nerves. *Biol. Bull. mar. biol. Lab., Woods Hole* **132**, 381–391.

ROBERTS, C. J., KROGSGAARD-LARSEN, P. AND WALKER, R. J. (1981). Studies on the action of GABA, muscimol and related compounds on *Periplaneta* and *Limulus* central neurons. *Comp. Biochem. Physiol.* **69C**, 7–11.

ROBINSON, T. N., LUNT, G. G., BATTENBY, M. K., IRVING, S. N. AND OLSEN, R. W. (1985). Insect ganglia contain [³H]flunitrazepam-binding sites. *Biochem. Soc. Trans.* **13**, 716–717.

ROBINSON, T., MACALLAN, D., LUNT, G. AND BATTERSBY, M. (1986). γ-Aminobutyric acid receptor complex of insect CNS: characterization of a benzodiazepine binding site. *J. Neurochem.* **47**, 1955–1962.

ROBINSON, T. N. AND OLSEN, R. W. (1988). GABA. In *Comparative Invertebrate Neurochemistry* (ed. G. G. Lunt & R. W. Olsen), pp. 90–123. London, Sydney: Croom Helm.

SCOTT, R. H. AND DUCE, I. R. (1987). Pharmacology of GABA receptors on skeletal muscle fibres of the locust (*Schistocerca gregaria*). *Comp. Biochem. Physiol.* **86C**, 305–311.

SHIMAHARA, T., PICHON, Y., LEES, G., BEADLE, C. A. AND BEADLE, D. J. (1987). Gamma-aminobutyric acid receptors on cultured cockroach brain neurones. *J. exp. Biol.* **131**, 231–244.

SIMMONDS, M. A. (1983). Multiple GABA receptors and associated regulatory sites. *Trends Neurosci.* **6**, 279–281.

TAKEUCHI, A. AND ONODERA, K. (1972). Effect of bicuculline on the GABA receptor of the crayfish neuromuscular junction. *Nature, New Biol.* **236**, 55–56.

WAFFORD, K. A., LUMMIS, S. C. R. AND SATTELLE, D. B. (1988). Block of an insect CNS 4-aminobutyric acid (GABA) receptor by cyclodiene and cyclohexane insecticides. *Pesticide Sci.* **24**, 338–339.

WALKER, R. J., JAMES, V. A., ROBERTS, C. J. AND KERKUT, G. A. (1981). Studies on amino acid receptors of *Hirudo, Helix, Limulus* and *Periplaneta. Adv. Physiol. Sci.* **22**, 161–190.

WALKER, R. J. AND ROBERTS, C. J. (1982). The pharmacology of *Limulus* central neurons. *Comp. Biochem. Physiol.* **72C**, 391–401.

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

WATSON, W. H. & AUGUSTINE, G. J. (1982). Peptide and amine modulation of the *Limulus* heart: a simple neural network and its target tissue. *Peptides* **3**, 485–492.