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ACTIONS OF PUTATIVE AMINO ACID NEUROTRANSMITTERS ON THE NEUROPILE ARBORIZATIONS OF LOCUST FLIGHT MOTONEURONES

BY F. DUBAS

Zoologisches Institut der Universität, Rheinsprung 9, CH-4051 Basel, Switzerland

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Summary

To characterize the receptors for putative amino acid neurotransmitters present on the dendritic arborizations of flight motoneurones in *Locusta migratoria*, the effects of pressure applications of glutamate, γ-aminobutyric acid (GABA), aspartate, taurine, glycine and cysteine were studied using an animal preparation in which neuropile intracellular recordings could be made during expression of the flight motor output. A majority of cells responded to glutamate, GABA, aspartate and taurine. At resting potential, glutamate and GABA caused, in different cells, a depolarization, a hyperpolarization or, in a few cells, a biphasic response, all accompanied by a decrease in the size of the evoked and spontaneous postsynaptic potentials (PSPs). At spiking threshold, the responses were always hyperpolarizing. Activation of a chloride conductance mediated the effects of both glutamate and GABA. In some cells, the response to glutamate or GABA desensitized during long-lasting applications, but in most cells the amplitude of the response did not decrease during applications lasting several minutes. Responses to aspartate and glutamate had identical reversal potentials and cross-desensitized. Responses to GABA and taurine had more negative reversal potentials and did not cross-desensitize with those elicited by glutamate or aspartate. Only a few neurones responded to applications of glycine or cysteine at resting potential; they responded with an inhibition of spiking at depolarized potentials. These data suggest that a variety of amino acid receptors are present on the neuropile arborizations of flight motoneurones.

Introduction

In vertebrates, amino acids are the main neurotransmitters at synapses in the central nervous system (CNS). They not only mediate normal synaptic transmission but may also be involved in the regulation of synaptic efficacy; for example, during development or during the learning process (Monaghan *et al.* 1989).

Key words: locust, neurones, amino acid neurotransmitters, GABA, L-glutamate, receptors, insects, *Locusta migratoria*.

In invertebrates, especially in arthropods, there is accumulating evidence that L-glutamate (glutamate) and γ-aminobutyric acid (GABA) are the main excitatory and inhibitory neurotransmitters at the neuromuscular junction (for reviews, see Shinozaki, 1988, and Robinson and Olsen, 1988). In the last few years, several lines of evidence have also suggested that certain amino acids, especially GABA, act as centrally released neurotransmitters. Using an antibody against GABA, Watson (1986), Watson and Burrows (1987), Tyrer *et al.* (1988) and Robertson and Wisniowski (1988) have found an abundance of cells containing GABA-like immunoreactivity in the thoracic ganglia of *Locusta* and *Schistocerca*, some of which are physiologically characterized and known to be inhibitory. Evidence for the role played by glutamate is indirect; since some glutamatergic motoneurones make central synapses (Watson and Burrows, 1982; Burrows *et al.* 1989), glutamate is likely to be the neurotransmitter at these synapses. Sombati and Hoyle (1984) showed that ionophoretic applications of glutamate in certain areas of the neuropile caused leg motoneurones to depolarize and spike. Glutamate-like immunoreactivity has been found in the central projections of some motoneurones and in interneurones (Bicker *et al.* 1988; Watson, 1988). No evidence has been presented for the presence of other amino acids, although the experiments of Hue *et al.* (1979) have suggested that taurine and GABA may block synaptic transmission at an identified synapse in the cockroach.

The pharmacological effects of amino acid neurotransmitters, including glutamate, GABA, aspartate and taurine, have also been studied on central neurones (Kerkut *et al.* 1969*a*,*b*; Pitman and Kerkut, 1970; Usherwood *et al.* 1980; Wafford and Sattelle, 1986; Neumann *et al.* 1987). These studies, made on insect neurone somata *in situ* or in culture, have demonstrated that nerve cell bodies are endowed with a variety of putative neurotransmitter receptors with pharmacological profiles different from those of equivalent vertebrate receptors.

A major problem inherent in the pharmacological studies of invertebrate neurones is that, while recordings and drug applications are made at the cell body, the synapses are located on the dendritic arborizations that branch off from the main neurite at a distance from the cell body (see, for example, Watson and Burrows, 1982). To characterize the receptors that mediate the synaptic interactions between neurones, it is necessary to study the effects of neurotransmitters on the neuropile arborizations, where the synapses are located, as did Sombati and Hoyle (1984) in their pioneering experiments on leg motoneurones.

For the work described here, an animal preparation has been used that allows intracellular recordings to be made in the neuropile arborizations of neurones in the thoracic ganglia of locusts during the expression of the flight output (Robertson and Pearson, 1982). Amino acids were applied to the dendritic arborizations of flight motoneurones (FMNs) in the neuropile. The goals of this study were: first, to determine whether the amino acids found to have an action on neurone somata also elicit responses in the neuropile and, second, to see how applications of amino acids in the neuropile interfere with the synaptic responses elicited during flight activity.

Materials and methods

Experiments were performed on laboratory-bred adult locusts (*Locusta migratoria*). The animal preparation was similar to that used by Robertson and Pearson (1982) and has been described previously (Dubas, 1990). The meso- and metathoracic ganglia were exposed dorsally and glued to an earthed metal plate. All peripheral nerves of the thoracic and abdominal ganglia were cut, with the exception of the mesothoracic N6 and the metathoracic N1, to avoid movement artefacts. The sheath covering the dorsal part of the metathoracic ganglion was then removed using bent insect pins, without touching the underlying nervous tissue. With the body cavity serving as an organ bath, the exposed ganglia were continuously superfused with saline (in $mmol\,l^{-1}$: NaCl, 155; KCl, 3; $CaCl_2$, 4; Hepes, 10; sucrose, 25; pH adjusted to 6.8 with NaOH) at a rate of $5–10\,ml\,min^{-1}$. In the experiments involving changes of extracellular ionic concentrations, where quick exchange of the solutions was necessary, the volume of the body cavity was reduced by building a Vaseline wall around the meso- and metathoracic ganglia.

Fictive flight was elicited by directing puffs of air onto the head and was monitored with an extracellular electrode in the dorsal longitudinal muscle (an indirect wing depressor muscle: muscle 112). Motoneurones to this muscle are found in both the meso- and metathoracic ganglia and run in the nerves left intact.

Intracellular impalements of neurones were made in their neuropilar segments in the metathoracic ganglion using thin-walled glass electrodes (10–25 MΩ) filled with $3\,mol\,l^{-1}$ potassium acetate or $2.5\,mol\,l^{-1}$ potassium chloride. Recordings obtained using electrodes filled with either electrolyte were not significantly different from one another. Flight motoneurones were easily identified by the very dorsal location of their arborizations and by their typical rhythmical activity during fictive flight. In a few cases, the impaled motoneurones were filled with Lucifer Yellow (Stewart, 1981) and viewed *in vivo* under laser illumination to determine their identity and visualize the impalement site. In general, however, no attempt was made to identify each motoneurone, and they were classified only as depressors or elevators depending on whether they fired synchronously or asynchronously with muscle 112. The Lucifer fills showed that impalements occurred in the main neurite or the finer dendrites. Intra- and extracellular recordings were amplified and stored, with concurrent drug-ejection stimuli, on magnetic tapes for subsequent analysis. Current was injected into the FMNs *via* the recording electrode using a bridge circuit, balanced when the electrode was in the cell. Experiments were performed at room temperature (20–28 °C).

Amino acids were pressure-applied to the desheathed surface of the ganglion from micropipettes positioned 10–50 μm from the intracellular electrode. Pressure-ejection pipettes were made of seven glass capillaries, glued together and pulled on a vertical Narishige puller. The common tip was broken back to a diameter of 2–10 μm and, after filling each channel, the assembly was connected to a system of seven pressure valves and checked for leakage in a drop of concentrated Neutral Red. Pressure pulses (35–70 kPa) of various durations were

triggered by a multichannel stimulator (A.M.P.I). The following compounds were tested: L-glutamate (sodium salt: Fluka), γ-aminobutyric acid (Sigma), L-aspartate (sodium salt: Sigma), L-taurine (Sigma), L-cysteine (hydrochloride: Sigma), L-glycine (sodium salt: Sigma). They were dissolved in the perfusion saline (10–100 mmol l^{-1}) or in a solution of appropriate ionic composition and the pH was adjusted to 6.8.

Results

Although the sheath normally wrapping the ganglion and forming a barrier between the extra- and intraganglionic media had been opened and removed from the dorsal surface in these experiments, the activity of the FMNs was identical, at rest and during fictive flight (Fig. 1A), to that recorded by Hedwig and Pearson (1984) from ganglia with intact sheaths. There was also good coordination between activity recorded in several different muscles on both sides of the body, suggesting that the flight neuronal network was intact. The resting potential (RP) of the FMNs was between −55 and −75 mV and spontaneous depolarizing or hyperpolarizing synaptic potentials of 1–5 mV were typical. The spiking threshold was at least 10–15 mV above RP.

A summary of the responses evoked by pressure applications of the various amino acids in the 52 preparations used for this series of experiments is given in Table 1. Some of the data concerning the inhibitory effect of glutamate have been the subject of a preliminary publication (Dubas, 1990), but they are presented here in detail to allow comparison with the actions of other amino acids.

Responses to glutamate

91% of the 191 FMNs tested responded to applications of glutamate and showed one of three responses at resting potential (RP): a pure depolarization (D response; 47% of the cells), a pure hyperpolarization (H response; 40%) or a biphasic response (B response: 4%) consisting of a depolarization superimposed

Table 1. *Summary of the cellular responses obtained at resting potential to pressure applications of various amino acids to the neuropilar arborizations of locust flight motoneurones*

Substance	Number of animals	Total number of cells	Cellular response 0 (%)	D (%)	H (%)	B (%)
Glutamate	52	191	17 (9)	91 (47)	75 (40)	8 (4)
GABA	14	44		20 (45)	21 (48)	3 (7)
Aspartate	6	20	2 (10)	16 (80)	2 (10)	
Taurine	13	47	6 (13)	12 (25)	29 (62)	
Glycine	6	30	11 (36)	13 (43)	6 (20)	
Cysteine	3	12	7 (58)	5 (42)		

0, no response; D, depolarization; H, hyperpolarization; B, biphasic response.

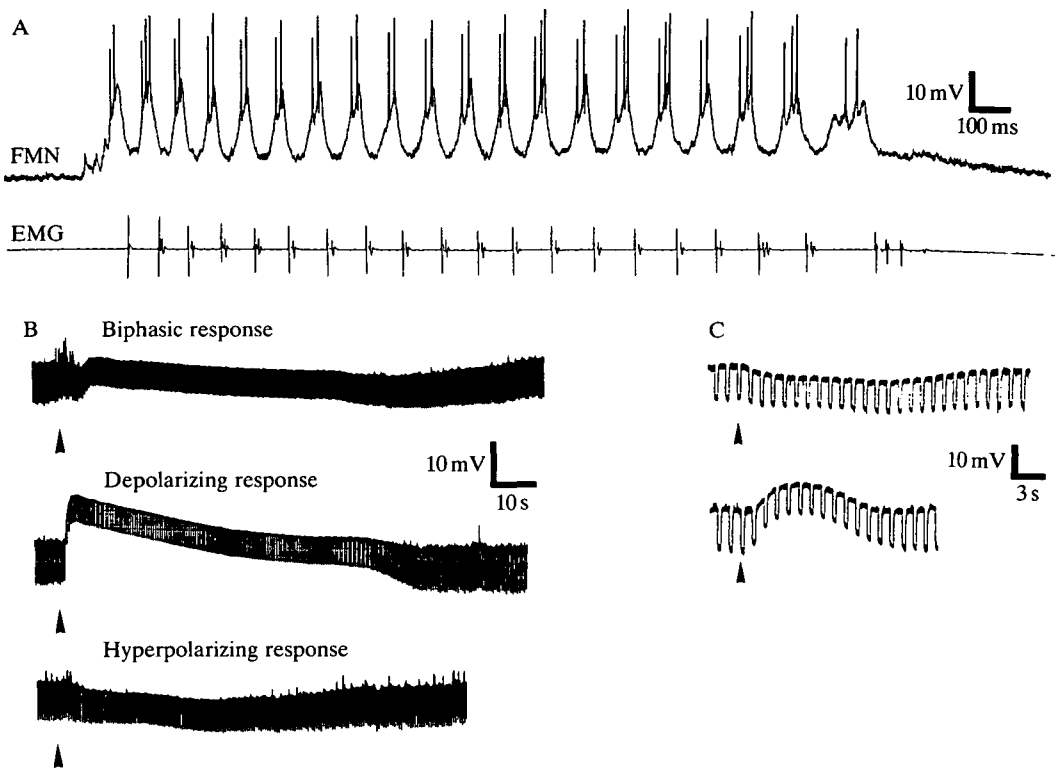

Fig. 1. (A) Flight motor output recorded intracellularly from an unidentified metathoracic elevator motoneurone (FMN) and extracellularly (EMG) from a depressor muscle (muscle 112: the dorsal longitudinal muscle). (B) Intracellular recordings of the effects of glutamate applications (50 ms pulse duration) on three different cells in the same preparation. Constant-current hyperpolarizing pulses (100 ms, 0.5 nA) were applied to measure membrane resistance. In this and the following figures, drug concentration in the pipette was 100 mmol l^{-1}, unless otherwise indicated. (C) Effects of GABA applications (100 ms) on two different FMNs of the same preparation. Upper trace: hyperpolarizing response; lower trace; depolarizing response. Hyperpolarizing current pulses: 300 ms, 0.5 nA.

on a hyperpolarization (Fig. 1B). The three types of responses were accompanied by an increase of membrane conductance and by a significant decrease in the amplitude of the spontaneous depolarizing or hyperpolarizing synaptic potentials (Figs 1B and 5). They could be observed both in elevator and depressor motoneurones and with electrodes filled with potassium chloride, potassium acetate or Lucifer Yellow.

As shown in Fig. 2, the three types of responses were still observed after synaptic transmission had been blocked by replacing the perfusion medium with a saline containing 20 mmol l^{-1} magnesium and 0 mmol l^{-1} calcium, indicating that they result from an action of glutamate directly on the FMNs. A further indication

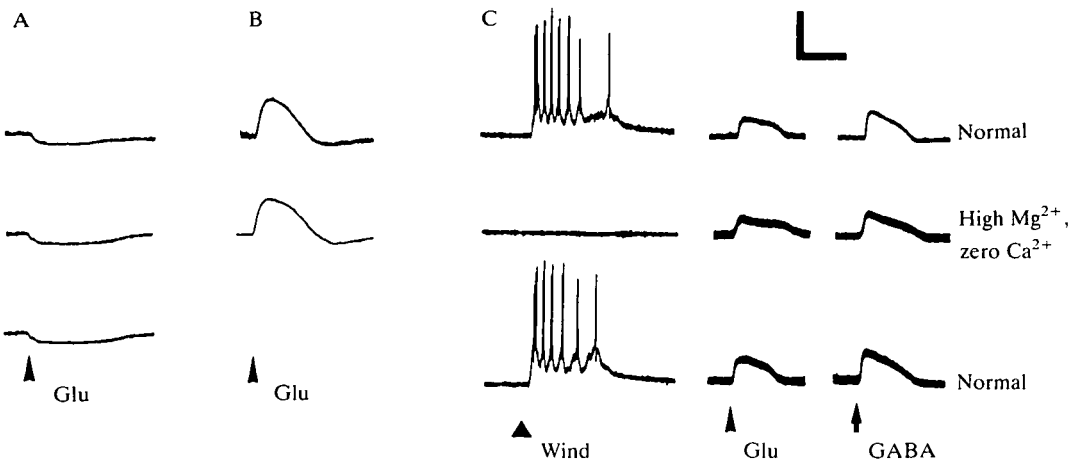

Fig. 2. The responses to applications of glutamate (Glu) (500 ms) or GABA (500 ms) are postsynaptic. Three cells are shown (A, B, C), in which glutamate or GABA elicited different types of responses. In cell C, the activity of the neurone during fictive flight is shown on the left. This activity was blocked in the high-magnesium, zero-calcium solution while responses to glutamate or GABA were still present. The responses to drug application remained unchanged after the return to normal solution and after the reappearance of the evoked synaptic activity (not shown in B). Scale bar, 10 mV/10 s for responses to drugs; 20 mV/500 ms for flight activity in C.

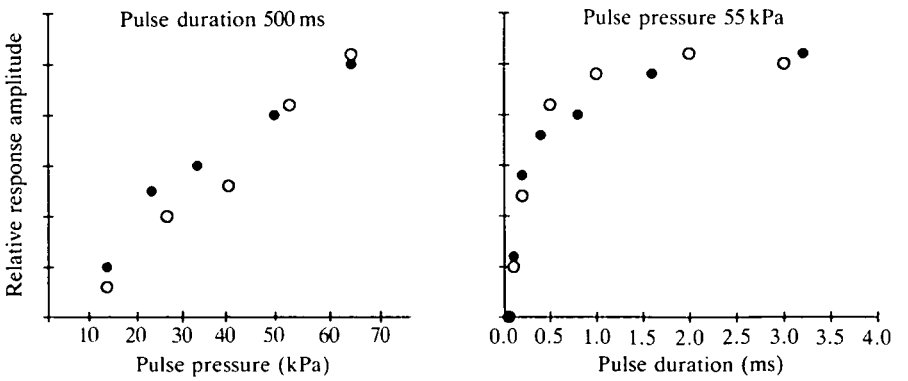

Fig. 3. Dose dependency of the responses evoked by pulses of glutamate (○) or GABA (●). The amplitudes of similar responses obtained with pulses of glutamate or GABA in different cells are plotted against pulse pressure or duration.

that the responses to glutamate are postsynaptic is that the amplitude of pure H or D responses in normal solution was dose-dependent, regardless of whether the pressure or duration of the application pulse was increased (Fig. 3). Larger doses of glutamate elicited an increase in the duration of the responses with a negligible increase in amplitude. Spikes were never elicited in FMNs, even in those showing D responses at RP.

Fading or reduction of the amplitude of the response to glutamate during long-lasting applications was examined in cells showing pure H or pure D responses. In some cells, applications of glutamate lasting several seconds caused a slow reduction of the H or the D responses (Fig. 4B). In other cells, the amplitude of the response evoked by glutamate did not decrease appreciably during applications lasting several minutes (Fig. 4C). Pulses of glutamate repeated at frequencies between 0.1 and 0.5 Hz elicited responses of slightly decreased amplitude. However, it was never possible to abolish the response totally

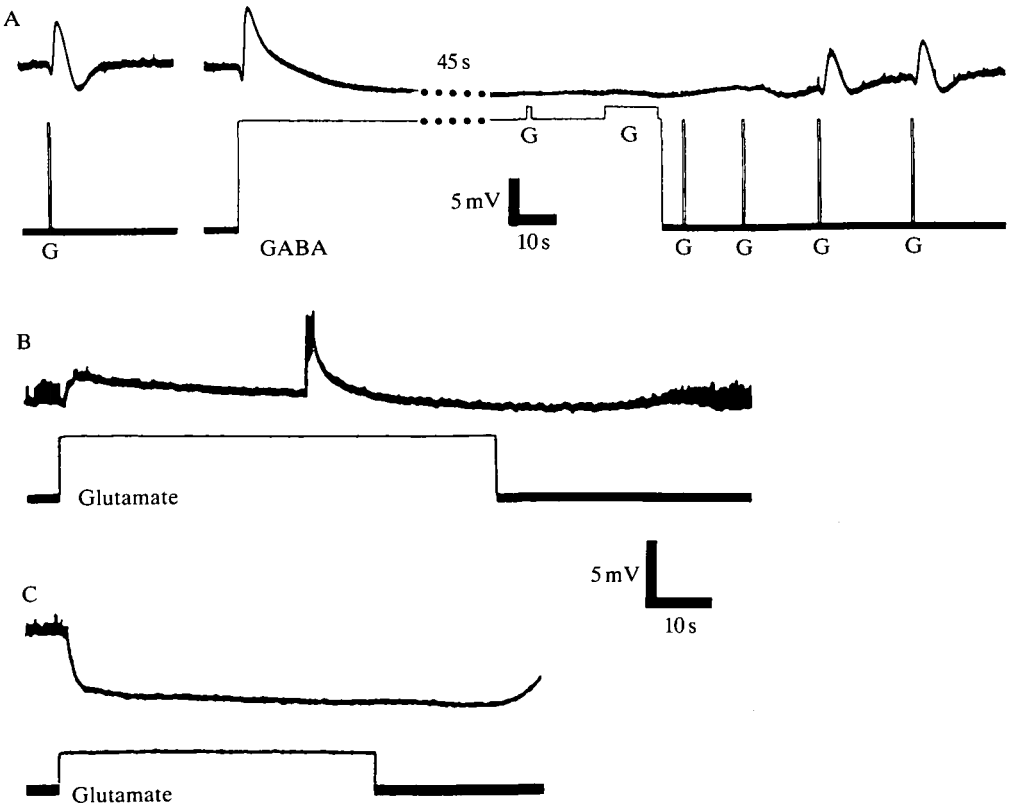

Fig. 4. Changes in membrane polarization evoked by long-lasting applications of glutamate or GABA. (A) A biphasic response was caused by application of GABA (300 ms). This response declined to zero during long-lasting application and, after a few minutes, no response could be elicited by further applications of GABA (G). The response to GABA returned within 30 s after the end of the long-lasting pulse. (B) A cell in which glutamate evoked a depolarizing response which faded during a long-lasting application. (C) Another cell in which glutamate evoked a hyperpolarizing response that did not desensitize. The PSPs in the middle of B represent subthreshold spontaneous flight activity. In each section, the upper trace is an intracellular recording and the lower trace is the application pulse. Note that the different amplitudes of the application pulses serve only to identify the active drug-ejection channel. They do not reflect pulse pressure.

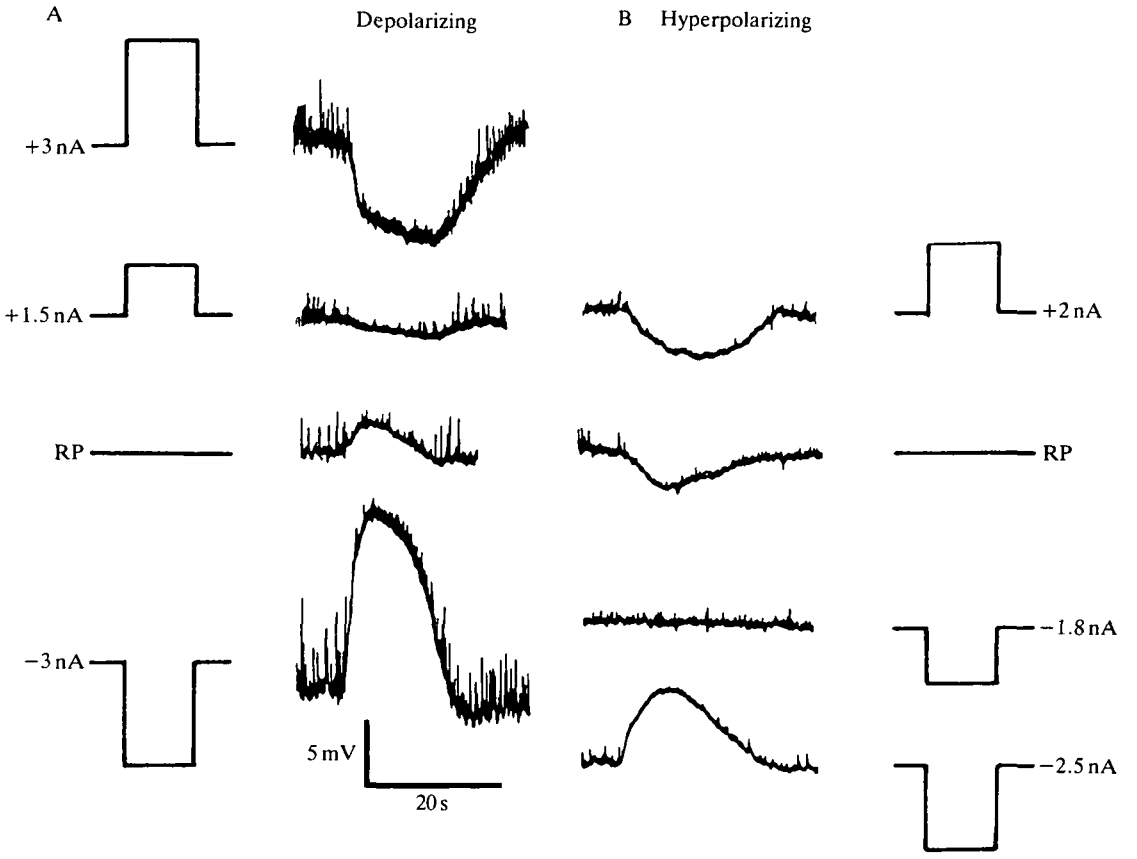

Fig. 5. Effect of membrane polarization on the amplitude of pure depolarizing (left) or hyperpolarizing (right) responses evoked by glutamate pulses (A, 500 ms; B, 1 s) in two different cells. The D response reversed at potentials more positive than RP, while the H response reversed at potentials more negative than RP. The cell in A also shows large synaptic potentials whose amplitude decreased during the response to glutamate and which increased with membrane hyperpolarization. In this and the following figures, a diagram of the current injected is placed next to each recording.

with repetitive pulses of glutamate, except when pulses fell during the peak of an already maximal response.

Although H, D and B responses could be recorded at RP in different FMNs, only hyperpolarization and inhibition of spiking resulted from glutamate applications on cells depolarized to their spiking threshold (Figs 5 and 6). To characterize the ionic conductances responsible for the different types of action of glutamate, the reversal potential of the responses and their behaviour in solutions of different ionic composition were examined. The reversal potential of the H response was always more negative than the resting potential (Fig. 5), while the reversal potential of the D response was more positive than RP but always more negative than the spiking threshold (Figs 5 and 6). The reversal potential could not

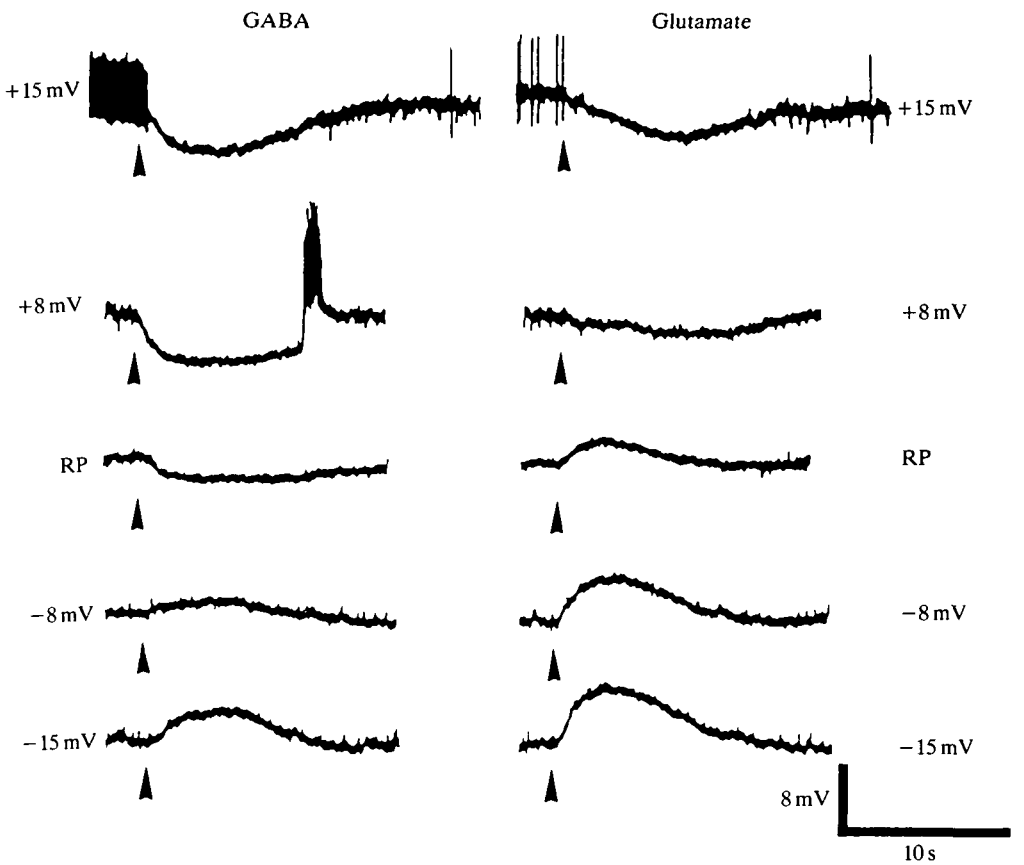

Fig. 6. Effects of glutamate and GABA in the same cell have different reversal potentials. In this cell, application of glutamate (10 mmol l^{-1}, 1 s) caused a D response at RP while the same dose of GABA caused an H response. When the cell was depolarized to spiking threshold, the D response reversed and both substances inhibited spiking. In this cell, only the approximate depolarization level was monitored.

be determined more accurately owing to an ineffective space clamp arising from the large size of the FMNs and the presence of an extensive dendritic tree. Thus, current injected from a single electrode is unlikely to be sufficient to achieve a uniform current clamp over more than the region of cell membrane localized around the point of impalement. The B responses recorded in some cells at RP became hyperpolarizing and monophasic when these cells were depolarized and they became depolarizing and monophasic when the cells were hyperpolarized. The presence of B responses at RP may indicate the existence of an underlying component of the glutamate response with a different reversal potential, but the nature of this effect is unknown.

Both D and H responses seemed to involve a chloride conductance. When the

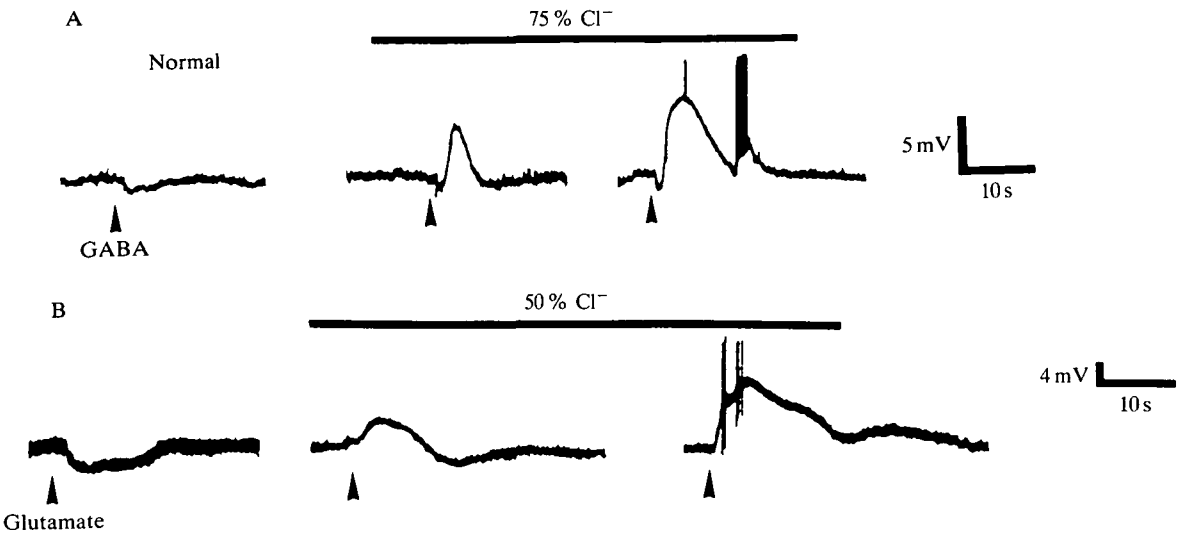

Fig. 7. Reduction of the extracellular chloride concentration produces a reversal of the responses to GABA (A) or glutamate (B). In the normal solution, an H response was elicited by GABA (30 ms) or glutamate (500 ms). In low-chloride medium (chloride substituted with gluconate), the response reversed, at least in part, and finally clipped spikes were elicited at RP. The burst of spikes following the response in low-chloride saline in the upper trace reflects spontaneous activity.

perfusion medium was replaced by a saline in which 50 % of the sodium chloride was substituted by sodium gluconate, the amplitude of the H response decreased and, after a few minutes, the H response reversed and became depolarizing (Fig. 7B). In contrast, the amplitude of D responses increased in the same solution. Intracellular injection of chloride ions had very little effect on the amplitude of the responses, presumably also because of the large size of the dendritic tree.

These experiments suggest that the pure H and D responses result from activation of the same ionic mechanism, a chloride conductance, which is always hyperpolarizing and inhibitory at spiking threshold but which can be depolarizing or hyperpolarizing at RP, depending on whether the RP of a particular cell is more negative or more positive than the reversal potential of the chloride conductance involved.

Responses to GABA

All of the 44 cells tested responded to pressure applications of GABA and showed either a pure H response, a pure D response or a biphasic response consisting of a superimposed depolarization and hyperpolarization. In different cells, the appearance and the time course of the biphasic responses were very different, the depolarization sometimes being triggered first or sometimes following a brief hyperpolarizing phase. All three responses were accompanied by an

increase in the membrane conductance and a reduction of the amplitude of the spontaneous synaptic potentials (Figs 1C and 4A).

The amplitude of the response to GABA was dose-dependent, regardless of whether the pulse pressure or duration was increased (Fig. 3). As was seen with glutamate, supramaximal applications of GABA resulted in a longer response duration. In a small number of cells, prolonged applications of GABA caused fading of the response (Fig. 4A), whereas in other cells the amplitude of the H or D response decreased only slightly over several seconds (Fig. 8). In a few cells, the amplitude of the change in membrane potential caused by a test pulse was attenuated following a long application of GABA and returned to its original value only several seconds after the end of the long GABA application (Fig. 4A).

When the FMNs were depolarized to their spiking threshold, GABA elicited only hyperpolarizations and inhibitions of spiking, since the D responses always reversed at membrane potentials more depolarized than RP.

The behaviour of the D and H responses elicited by GABA in solutions of reduced chloride concentration was the same as that elicited by glutamate: H responses reversed within a few minutes (Fig. 7A), whereas the amplitude of D responses increased in solutions of lowered chloride concentration, sometimes reaching spiking threshold. In some cells, like the one shown in Fig. 7A, only part of the H response reversed, suggesting that another effect, relying on another ionic conductance, may also participate in the membrane potential changes. This additional effect (also suggested by the presence of B responses at RP) may be mediated by a potassium conductance, but no clear evidence could be found for the involvement of potassium ions.

Comparison between responses evoked by glutamate and GABA

It is clear from the data presented above that glutamate and GABA have similar actions on the FMNs: both neurotransmitters cause depolarizations or hyperpolarizations at RP, but at spiking threshold both substances are inhibitory. A chloride conductance seems to be involved in both effects. However, applications of glutamate and GABA to the same cells indicated that the mechanisms mediating the effects of these two substances on the FMNs are distinct.

Although glutamate and GABA elicited similar responses on certain FMNs at RP (Fig. 2C), the reversal potential of the responses triggered by GABA was more negative than that of the response to glutamate. Further, in many cells, GABA elicited an H response at RP, while glutamate caused a D response (Fig. 6).

In the FMNs, repetitive pulses of glutamate or GABA never caused a complete abolition of the response to one or the other substance, so that desensitization experiments could not be used to determine whether GABA and glutamate act on the same receptors (as suggested by Franke *et al.* 1986). Instead, long-lasting applications of one compound were used to saturate its receptors and test pulses of the other substance were applied when no further response to the first substance could be elicited (applied from a third channel). Fig. 8 shows two such expe-

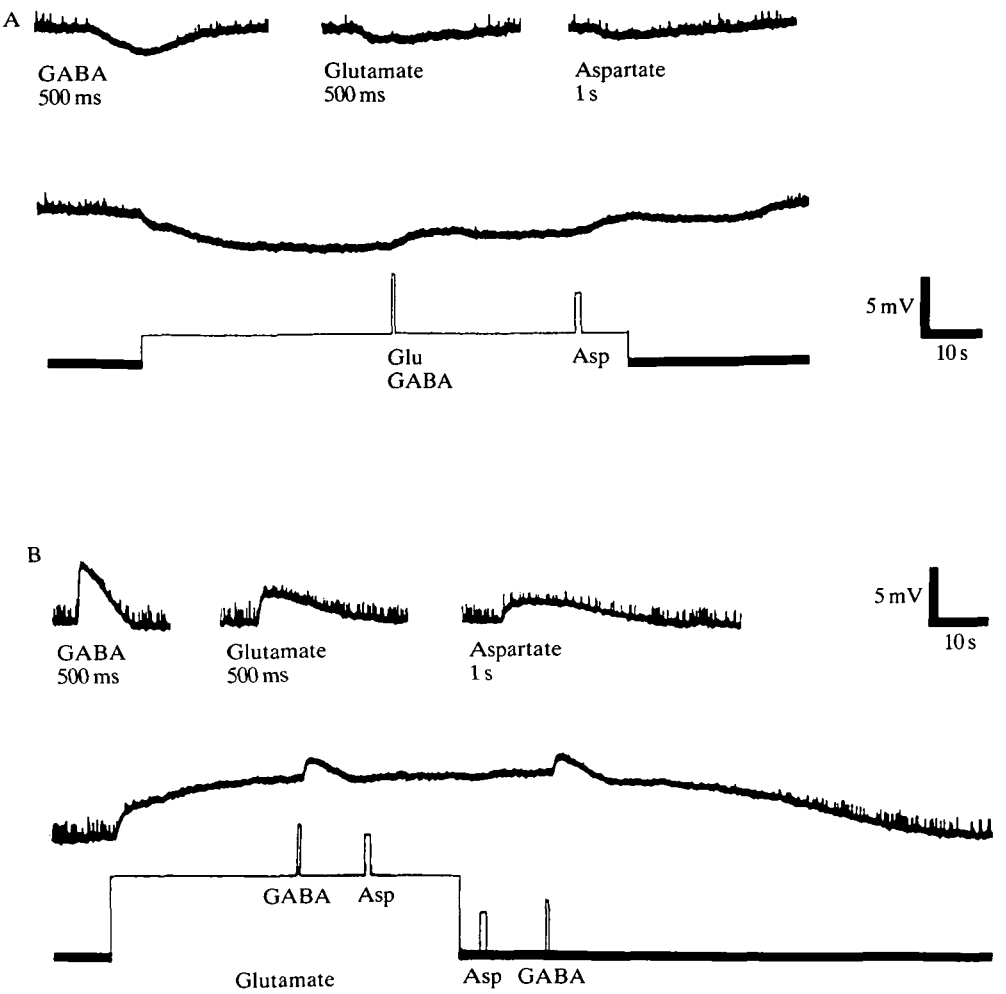

Fig. 8. Long-lasting application of GABA or glutamate (Glu) does not abolish the response to the other substance. The responses elicited by GABA, glutamate and aspartate (Asp) in two different cells are shown in A and B. (A) During a long-lasting application of GABA, responses to glutamate and aspartate were not abolished. Note that the responses to glutamate and aspartate elicited during the pulse of GABA are reversed, because the cell is hyperpolarized, presumably below the reversal potential of the glutamate effect. Conversely (B), a long-lasting application of glutamate saturated the receptors to glutamate. During this pulse, responses to aspartate, but not those to GABA, were abolished. In each section, the upper trace is the intracellular recording and the lower trace is the application pulse. Note that the different amplitudes of the application pulses serve only to identify the active drug-ejection channel.

riments: during a long-lasting pulse of glutamate, a response to GABA could still be elicited and, conversely, the response to glutamate was not abolished in the presence of saturating levels of GABA. Taken together, the above experiments

suggest that, although GABA and glutamate cause similar responses on the FMNs, they act *via* distinct receptors and conductances.

Effect of glutamate and GABA on evoked synaptic activity

Both glutamate and GABA were effective in decreasing the amplitude of the synaptic activity evoked during flight, so that, with carefully adjusted doses of glutamate or GABA, it was possible to reduce the amplitude of the evoked synaptic potential below the spiking threshold. The rhythmical synaptic potentials elicited by the flight oscillator were still evident in the impaled neurone, indicating that the flight oscillator was not inhibited, but they were not large enough to reach spiking threshold (Fig. 9B,C).

The following two lines of evidence suggest that this inhibitory effect of glutamate and GABA on evoked activity is not caused by receptor desensitization. First, the amplitude of both excitatory and inhibitory synaptic potentials, spontaneous or evoked, was decreased during applications of glutamate or GABA. Second, the amplitude of the depolarization caused by a test pulse of acetylcholine (ACh) was also decreased when a pulse of glutamate or GABA immediately preceded ACh (Fig. 9A). Thus, the effects of GABA and glutamate on evoked or spontaneous synaptic potentials are best explained by a 'shunt' of the potential changes caused by the opening of a large number of ion channels.

Responses to aspartate

The effect of aspartate was studied in 20 FMNs (Table 1). In all of these cells, the effect of aspartate was the same as that of glutamate. However, applications of aspartate and glutamate to the same cells, at the same concentration in the pipette, indicated that aspartate was slightly less potent than glutamate, especially in eliciting H responses. The reversal potential of the membrane polarizations caused by the two compounds were identical and differed from the reversal potential of the responses to GABA (Fig. 10).

During prolonged applications of glutamate, responses to aspartate were abolished while those to GABA were not. In contrast, during application of GABA, responses to both glutamate and aspartate could be observed (Fig. 8). This supports the idea that aspartate has an agonist effect on glutamate receptors.

Responses to taurine

Taurine was tested on 47 FMNs (Table 1). In all cells tested, taurine always caused a hyperpolarization and a clear inhibition of spiking at depolarized potentials, although the response at RP could be depolarizing or hyperpolarizing (Figs 10 and 11). In a few cells, the response to taurine at RP reached 5–7 mV and was accompanied by a clear resistance decrease, but in most cells it was very small. In three cells that showed no response to taurine at RP, spiking was inhibited when the cells were depolarized to their spiking threshold.

In all cells tested, the reversal potential of the action of taurine was more negative than the reversal potential of the effect of glutamate. Taurine and GABA

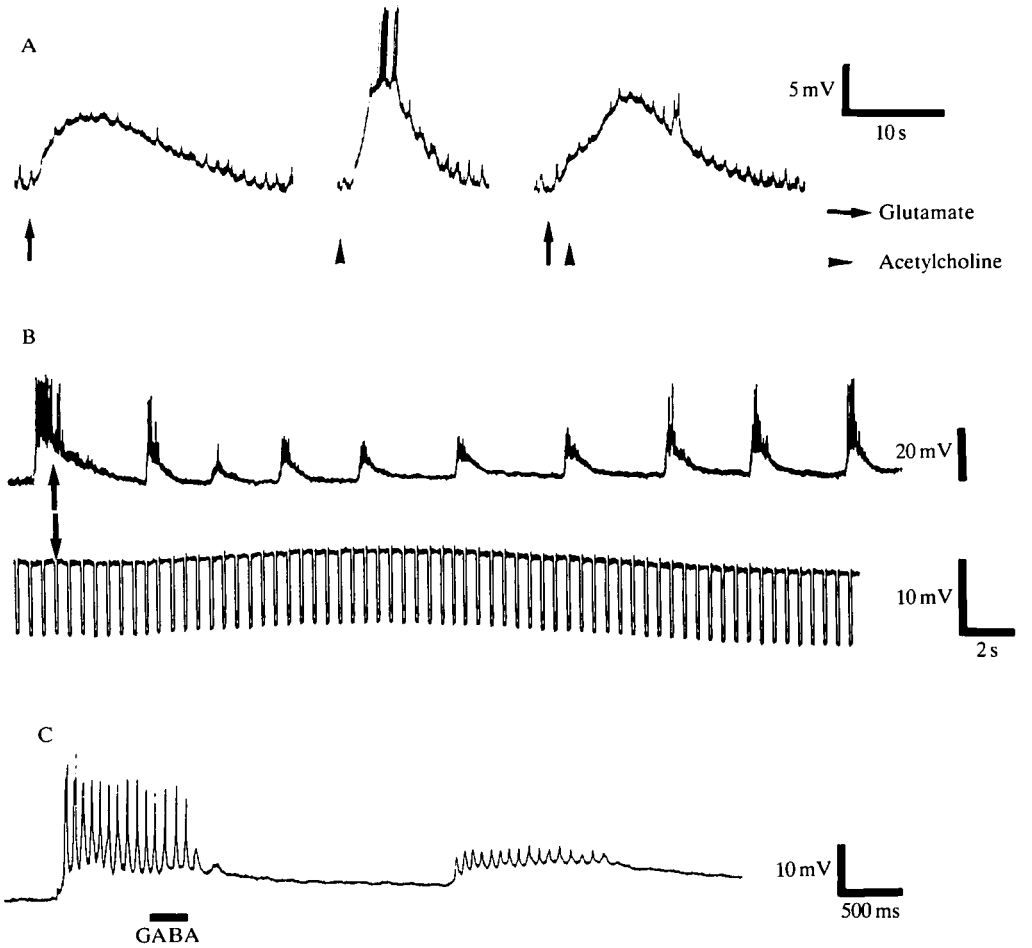

Fig. 9. Applications of GABA or glutamate caused a decrease in the amplitude of membrane polarization changes in response to other neurotransmitters. (A) In this cell, application of glutamate (1 s) evoked a D response while acetylcholine (ACh) (10 mmol l^{-1}, 40 ms) caused a larger depolarization and spiking. When the same pulse of ACh was applied 1 s after the end of the glutamate pulse, the response to ACh failed to reach spiking threshold. (B) Application of glutamate (100 ms) during fictive flight activity elicited by wind caused a reduction in the amplitude of the evoked depolarizations and prevented the cell from reaching spiking threshold. The lower trace in this section shows the response evoked in the same cell by the same dose of glutamate in the absence of fictive flight activity. Constant-current pulses (100 ms, 0.7 nA) were injected to show membrane resistance changes. (C) Application of GABA (200 ms) during fictive flight activity in another cell also produces a decrease of the amplitude of the evoked synaptic potentials. Note the difference in time scale between B and C.

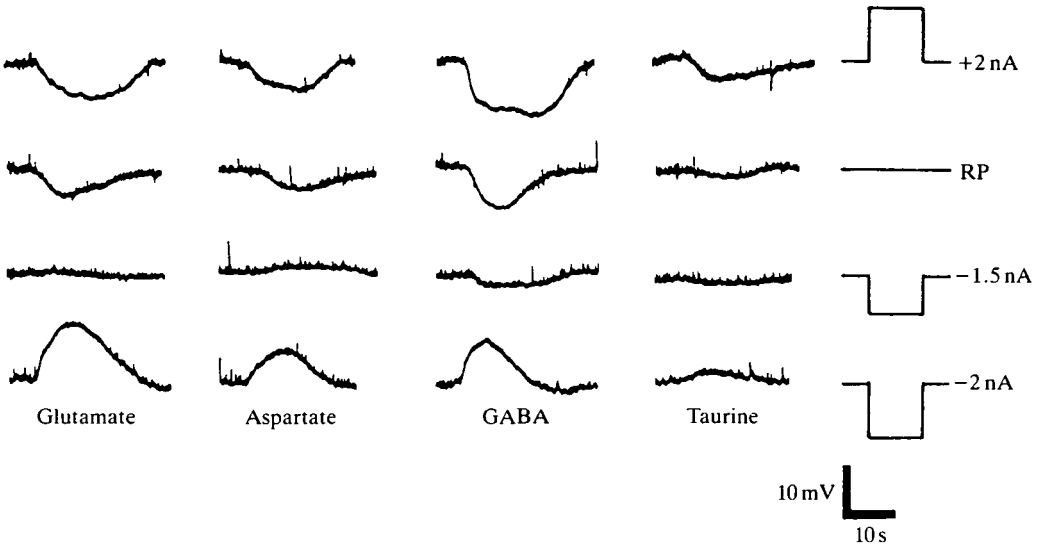

Fig. 10. Comparison of the reversal potentials of responses to pulses of glutamate (1 s), aspartate (1 s), GABA (500 ms) and taurine (500 ms) in a single cell. Each substance caused an H response at resting potential, but when the cell was hyperpolarized just below RP, glutamate and aspartate caused a D response whereas GABA and taurine still elicited a small H response. At a more negative potential, a depolarization was evoked by each substance. Drug concentration was 100 mmol l^{-1}.

elicited responses with reversal potentials that were not measurably different. Since the response to taurine was usually small at RP, it was not possible to see whether saturation of GABA receptors abolished the response to taurine.

Responses to glycine and cysteine

Only a few cells responded to glycine or cysteine at RP (Table 1). However, in a majority of the cells tested, glycine and cysteine elicited a hyperpolarizing response and inhibited spiking when the cells were depolarized to their spiking threshold (Fig. 11). Conductance changes could not be detected but there was a clear reduction in the amplitude of synaptic potentials with both glycine or cysteine.

Discussion

The data presented here demonstrate that a variety of receptors for amino acid neurotransmitters are present on the neuropilar segment of the FMNs and that the cellular effects mediated by these receptors can be studied in semi-intact preparations with intracellular electrodes inserted in the neuropile arborizations. Using this preparation, it is now possible to study the pharmacology of the receptors mediating synaptic interactions and thus identify the neurotransmitters involved.

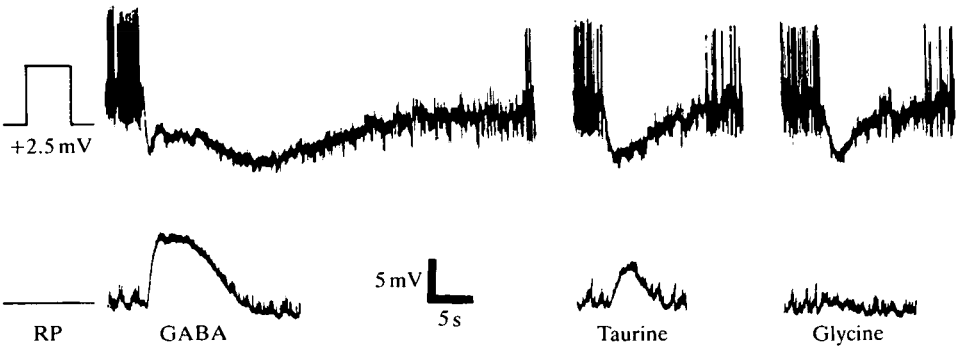

Fig. 11. Depolarizing responses caused by GABA (500 ms), taurine (800 ms) and glycine (500 ms) in the same cell, at RP, become hyperpolarizing when the cell is depolarized to its spiking threshold. Note the biphasic hyperpolarization elicited by GABA at depolarized potentials.

It must be pointed out, however, that, as extrasynaptic neurotransmitter receptors exist on the somata, far away from the synaptic sites, extrasynaptic receptors may also be present in the neuropile, closer to the synapses. In insect muscles, Lea and Usherwood (1973) and Cull-Candy (1976) have demonstrated convincingly the presence of excitatory and inhibitory glutamate receptors in the extrasynaptic muscle membrane, in addition to the synaptic excitatory glutamate receptors that mediate neuromuscular transmission (Usherwood, 1981). The function of the extrasynaptic glutamate receptors in insect muscle is so far unknown. In the experiments described here on the FMN neuropile, it is not clear whether the receptors mediating the effects of the various amino acids are synaptic or extrasynaptic. However, activation of the receptors for glutamate or GABA in the neuropile is sufficient to decrease the amplitude of the synaptic activity evoked during flight below the spiking threshold (Fig. 9). Thus, whether of not they are synaptic, the receptors for GABA and glutamate present in the neuropile can modulate the efficacy of synaptic inputs. This action could be mediated by membrane conductance changes.

The results presented here indicate that the action of glutamate on the neuropile arborizations of FMNs is inhibitory, although D and H responses can be recorded at RP in different cells. This effect is mediated, at least in part, by a chloride conductance. The present experiments further suggest that aspartate is a probable agonist on the glutamate receptors, but that GABA is not. Comparable results were obtained by Wafford and Sattelle (1989), working on the soma of a cockroach leg motoneurone *in vivo* where glutamate activates a chloride conductance with a reversal potential of −82 mV which can also be activated by aspartate but not by GABA. Inhibitory responses to applications of glutamate have been recorded from non-identified somata isolated from adult locusts or from cockroach embryos (Usherwood *et al.* 1980; Horseman *et al.* 1988), as well as from neurones of molluscs (Walker, 1976; Ikemoto and Akaike, 1988), crustaceans (Roberts and

Walker, 1982; Marder and Paupardin-Tritsch, 1978) and annelids (Walker *et al.* 1981; Mat Jais *et al.* 1983). In view of the data presented here, it is possible that receptors mediating the inhibitory effect of glutamate will be found in the neuropile arborizations of many other invertebrate neurones, possibly indicating a modulatory role of glutamate.

The inhibitory effect shown here to result from applications of glutamate on the FMN neuropile contrasts with the findings of Sombati and Hoyle (1984), who showed that glutamate ionophoresed in the neuropile could cause identified leg motoneurones to depolarize and spike. Since these authors made no effort to block synaptic transmission, it is possible that, in their preparation, glutamate had a presynaptic inhibitory effect. However, Burrows *et al.* (1989) have recently identified physiologically a chemically mediated, central, excitatory synapse between glutamatergic leg motoneurones. Thus, the presence of synaptic excitatory glutamate receptors on at least some of the leg motoneurones is to be expected. In contrast, FMNs are not known to receive excitatory inputs from other FMNs (see Robertson and Pearson, 1982) and thus glutamatergic excitatory synapses may be totally lacking. Preliminary data obtained using the same preparation suggest that acetylcholine, which is thought to be released by sensory afferents (see Pitman, 1985; Lutz and Tyrer, 1988), is a powerful excitatory transmitter on the FMNs.

Both in isolated insect neurone somata (Usherwood *et al.* 1980; Lees *et al.* 1987; Neumann *et al.* 1987) and in somata *in situ* (Kerkut *et al.* 1969*b*; Wafford and Sattelle, 1986; Pinnock *et al.* 1988; Sattelle *et al.* 1988), the main response to GABA is a chloride-mediated inhibition with a reversal potential close to RP. This response is similar to the pure inhibitory response recorded in the FMN neuropile. However, in the FMN neuropile, as in neurone somata (Wafford and Sattelle, 1986; Lees *et al.* 1987), GABA can elicit a variety of responses, suggesting that different GABA-receptor-linked mechanisms could be present. A variety of GABA receptor types with different pharmacological profiles (Wafford and Sattelle, 1986; Lees *et al.* 1987; Benson, 1988) have been characterized in different insect nervous system preparations.

The physiological and immunocytological evidence available suggests that at least some of the inhibitory interneurones of the flight pattern generator are GABAergic and that the PSPs they trigger in postsynaptic cells are blocked by picrotoxin (Robertson and Pearson, 1985; Robertson and Wisniowski, 1988). Some of these interneurones are known to contact the FMNs directly (Robertson and Pearson, 1983). It may be that, as for glutamate, there are synaptic and extrasynaptic GABA receptors and that these receptors have different pharmacological properties. Interestingly, Robertson and Wisniowski (1988) found at least one set of known inhibitory interneurones that did not contain GABA.

Applications of glutamate and GABA on the same cells show that the effects of both neurotransmitters are mediated, at least in part, by chloride conductances, but that the effects triggered by GABA usually have a reversal potential more negative than that of the responses caused by glutamate. This indicates that

another conductance must be involved in one of the two responses, but this mechanism has not been elucidated.

The effects of glycine, cysteine and taurine on the FMN neuropile are identical to their actions on the neurone somata (Usherwood *et al.* 1980; Giles and Usherwood, 1985; Wafford and Sattelle, 1986). In both cases, it is possible that these amino acids do not act on specific receptors but are agonists on receptors for either GABA or glutamate. However, since in the bee brain (Schäfer *et al.* 1988) there is evidence that taurine-like immunoreactivity is present in a distinct neuronal population and may play a role as a neurotransmitter, the possibility that at least taurine is a true neurotransmitter in locust thoracic ganglia cannot be excluded.

In summary, it is shown that the neuropile arborizations of the FMNs possess a variety of receptors for amino acids, mediating cellular responses similar to those on the somata. Further pharmacological data will be necessary to characterize the neuropile amino acid receptors and determine the role played by the different types of receptors in synaptic integration.

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