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EXCITATORY AMINO ACID RECEPTORS IN *XENOPUS* EMBRYO SPINAL CORD AND THEIR ROLE IN THE ACTIVATION OF SWIMMING

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SUMMARY

1. Bath application of *N*-methyl-D-aspartate (NMDA), kainate or quisqualate to *Xenopus* embryos depolarized spinal cord motoneurones and reduced their input resistance in both normal salines and salines containing 20 mM-Mn^{2+} and 0·5 mM-Ca^{2+}, or 2×10^{-6} M-tetrodotoxin. This suggests that motoneurones possess all three types of excitatory amino acid receptor.

2. These receptors have similar specificities to excitatory amino acid antagonists as those occurring in adult frog and cat spinal cords.

3. Application of 30–40 μM-NMDA or 5–6·5 μM-kainate to the medium bathing spinalized embryos can cause a sustained patterned motor output similar to that of swimming evoked by natural stimulation of intact animals. At these concentrations NMDA and kainate depolarized motoneurones by 19·0 ± 1·80 (mean ± S.E. of mean) and 18·0 ± 2·00 mV respectively and decreased their input resistance by 23·0 ± 2·82 % and 24·0 ± 3·46 %. These changes are similar to those associated with the tonic excitation which motoneurones receive during naturally evoked swimming.

4. Bath application of 5–8 μM-quisqualate to spinal embryos can also cause a sustained motor output. However, this was different to that evoked by NMDA and kainate and was inappropriate for swimming.

5. When applied to intact animals during swimming both 2–3 mM-*cis*-2,3-piperidine dicarboxylic acid (PDA) and 0·5 mM-γ-D-glutamylglycine (DGG) selectively blocked the tonic excitation of motoneurones and in doing so abolished the motor output of the spinal cord. 50–200 μM-2-amino-5-phosphonovaleric acid reduced the tonic excitation but to a lesser extent than either PDA or DGG. The tonic excitation of motoneurones which occurs during swimming therefore appears to be mediated via an endogenous excitatory amino acid transmitter which acts on NMDA and kainate receptors.

INTRODUCTION

In many animals brief sensory stimulation can evoke long lasting locomotor activity. In both vertebrates and invertebrates, the neurones which generate the underlying motor pattern are thought to be activated by a sustained input, possibly

a background of tonic excitation, originating from within the central nervous system (Grillner, 1975; Kupferman & Weiss, 1978).

Indirect evidence for tonic excitation comes from experiments in vertebrates where unpatterned electrical stimulation of the rostral spinal cord or hind brain (e.g. Shik & Orlovsky, 1976; Grillner, 1975; Lennard & Stein, 1977) or the application of pharmacological agents (Forssberg & Grillner, 1973; Poon, 1980) can give rise to a patterned motor output appropriate for a particular locomotion. There is also direct evidence that tonic excitation is present during rhythmic locomotor activity. In *Xenopus* embryos the phasic activity of spinal cord motoneurones (Soffe & Roberts, 1982*a*) and interneurones (Clarke, Roberts & Soffe, 1983) is superimposed on a tonic level of depolarization about 10–25 mV above the resting potential. In the sea slug *Tritonia* neurones of the swimming pattern generator also appear to receive sustained excitation during locomotion (Lennard, Getting & Hume, 1980).

The excitatory amino acids glutamate and aspartate have excitatory effects on many types of vertebrate neurone (Curtis & Watkins, 1963). The bath application of excitatory amino acids can elicit a rhythmic motor output appropriate for swimming in the lamprey (Poon, 1980; Grillner, Maclellan, Sigvardt, Wallen & Wilen, 1981). Recently Watkins & Evans (1981) have defined three distinct excitatory amino acid receptors, named after their principal agonists *N*-methyl-D-aspartate (NMDA), kainate and quisqualate respectively.

Pharmacological antagonists specific for the excitatory amino acids have been used to demonstrate that the excitation following dorsal root stimulation of adult frog and cat spinal cords is mediated largely via transmitters acting at excitatory amino acid receptors (Davies, Evans, Francis, Jones & Watkins, 1980; Davies & Watkins, 1981; Watkins, Davies, Evans, Francis & Jones, 1981).

The present paper shows that some excitatory amino acid agonists can evoke a motor output from the *Xenopus* embryonic spinal cord similar to that of naturally evoked swimming, and that the excitatory amino acid receptors present seem similar in their specificities to pharmacological agents to those in the adult frog and cat spinal cords. By using excitatory amino acid antagonists we have shown that the tonic excitation received by motoneurones during naturally evoked swimming is mediated via the release of a transmitter which acts at excitatory amino acid receptors. This gives an opportunity for the first time in a simple system, for the full characterization of both the pre- and post-synaptic elements of a synapse using an excitatory amino acid neurotransmitter.

METHODS

Stage 37–38 *Xenopus* embryos (Nieuwkoop & Faber, 1956) were removed from their egg membranes, paralysed in 10^{-4} M-tubocurarine chloride (Sigma) and held down on their side on a Sylgard table between two micropins inserted through the notochord. For extracellular motor nerve recordings, the skin was peeled back, using fine mounted needles, to reveal the myotomes. Glass suction electrodes were then placed on the intermyotome clefts to record the activity of motoneurone axons (see Kahn & Roberts, 1982*b*). In order to make intracellular recordings the myotomes overlying the spinal cord were removed (Fig. 1).

Neurones in the ventral half of the spinal cord could then be impaled with electrodes filled with 3 M-potassium acetate and having resistances of 100–160 MΩ. The recordings made in this study were almost certainly from motoneurones. Motoneurones are the only superficial cells in the ventral

half of the spinal cord (Roberts & Clarke, 1982) and have been identified by dye injection through micro-electrodes (Soffe & Roberts, 1982*a*). The cells which were penetrated had physiological properties identical to those of motoneurones identified by dye injection through micro-electrodes (Soffe & Roberts, 1982*a*). In seven cases it was possible to evoke one-for-one constant-latency ventral root spikes by intracellular stimulation of these ventral neurones, providing physiological evidence that they were indeed motoneurones. Conventional recording and amplification techniques were used, the results being stored on magnetic tape and permanent records made using a transient recorder (Datalab DL902) and an *X–Y* plotter. This work is based on intracellular recordings made from 135 motoneurones in 108 animals.

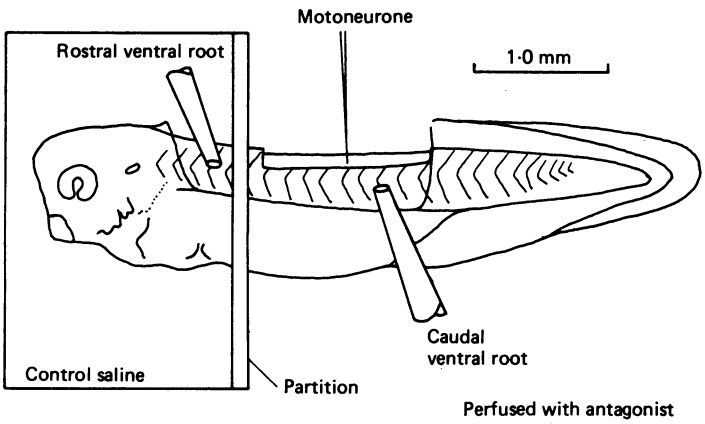

Fig. 1. The partitioned preparation. The skin of a stage 37–38 *Xenopus* embryo has been removed to reveal the myotomes, those myotomes overlying the spinal cord having been removed to allow intracellular recordings to be made. The partition (indicated) isolates the head compartment from the rest of the bathing medium.

The preparation table could be rotated about its long axis for dissection and recording and lay in a bath (volume 2 ml) which was continually perfused (10 ml per minute) with frog Ringer solution (composition: NaCl, 115 mM; KCl, 2·5 mM; $CaCl_2$, 5 mM; $NaHCO_3$, 2·4 mM; pH 6·8–7·2) containing 5×10^{-5} M-tubocurare. The perfusion could be switched to saline containing pharmacological agents in addition to the tubocurare. These agents, *N*-methyl-D-aspartate (NMDA), quisqualate, 2-amino-5-phosphonovalerate, *cis*-2,3-piperidine dicarboxylic acid and γ-D-glutamylglycine were obtained from Cambridge Research Biochemicals, while kainate and tetrodrotoxin (TTX) were obtained from Sigma.

Partitioned preparation

Once the embryo had been prepared for intracellular recording it was moved and re-pinned so that the head half poked into a small three-sided compartment. The fourth side of the compartment, a piece of acetate sheet with a notch cut in it, was then slid into place over the animal, the seal being made as complete as possible by squirting Vaseline around the edges of the partition. This isolated the medium surrounding the head from the rest of the bath which could be superfused with a saline containing an antagonist.

Abbreviations

APV	2-amino-5-phosphonovaleric acid
DGG	γ-D-glutamylglycine
NMDA	*N*-methyl-D-aspartate
PDA	*cis*-2,3-piperidine dicarboxylic acid
TTX	tetrodotoxin

RESULTS

Fictive swimming in intact embryos

Natural stimulation of intact *Xenopus* embryos will result in prolonged swimming episodes (Kahn, Roberts & Kashin, 1982). During this activity ventral root spikes recorded from the left and right sides alternate, the one being mid-cycle to the other

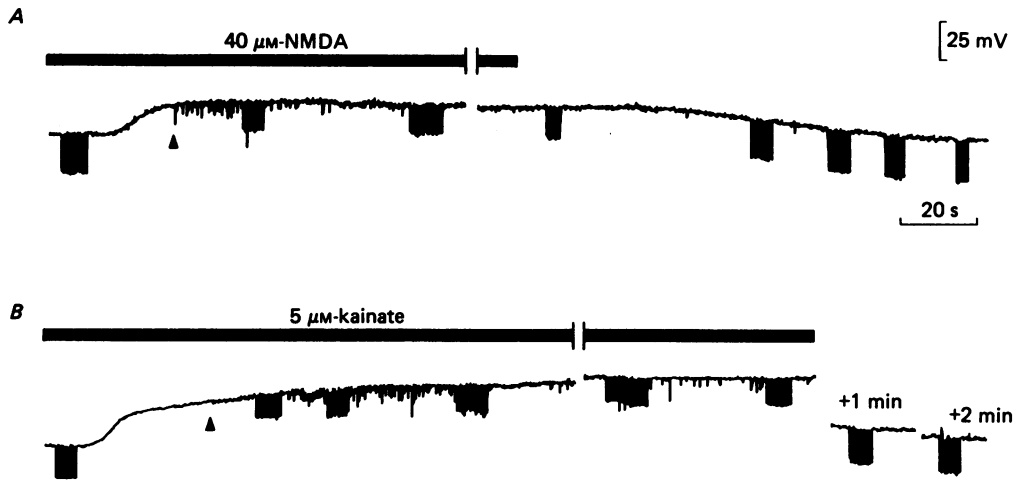

Fig. 2. Slow intracellular records of the effect of *A*, 40 μM-NMDA and *B*, 5 μM-kainate. Both produce a steady depolarization of membrane potential and lower input resistance (seen as a decrease in the amplitude of hyperpolarizing constant current pulses). Note that the onset of the depolarization in this case was followed by a barrage of i.p.s.p.s (arrowed). There are breaks of 42 s in *A* and 58 s in *B* during agonist application. In this and all subsequent Figures positive in the vertical scale is upwards.

with a cycle period of 40–125 ms (Kahn & Roberts, 1982*b*). Motoneurones fire spikes in phase with the ipsilateral ventral root spikes and receive a mid-cycle i.p.s.p. in phase with the contralateral ventral root spike (Roberts & Kahn, 1982; Soffe & Roberts, 1982*b*; see Fig. 3*A*). The phasic potentials seen in motoneurones are superimposed upon a tonic level of depolarization about 10–30 mV above the resting potential (Roberts & Kahn, 1982; and Fig. 3*A*).

Spinal embryos

Mechanical stimulation of the skin of spinal embryos results in only a brief flutter of the body (Kahn & Roberts, 1982*a*). Intracellular recordings show that there is an absence of spontaneous excitatory motoneurone activity in spinal embryos and also that, following stimulation of the skin, the tonic excitation is not sustained for more than about 300 ms (N. Dale & S. R. Soffe, unpublished observation). The spinal preparation is therefore ideal for examining whether excitatory amino acid agonists can activate the spinal cord to produce the swimming pattern in a sustained manner.

Agonist application in spinal embryos

The results in this section are from fifty experiments with extracellular recordings and from thirty-seven intracellular recordings from thirty-seven embryos. The addition of 0·5–0·75 mM-L-glutamate, 50–75 μM-NMDA or 8–10 μM-kainate to the

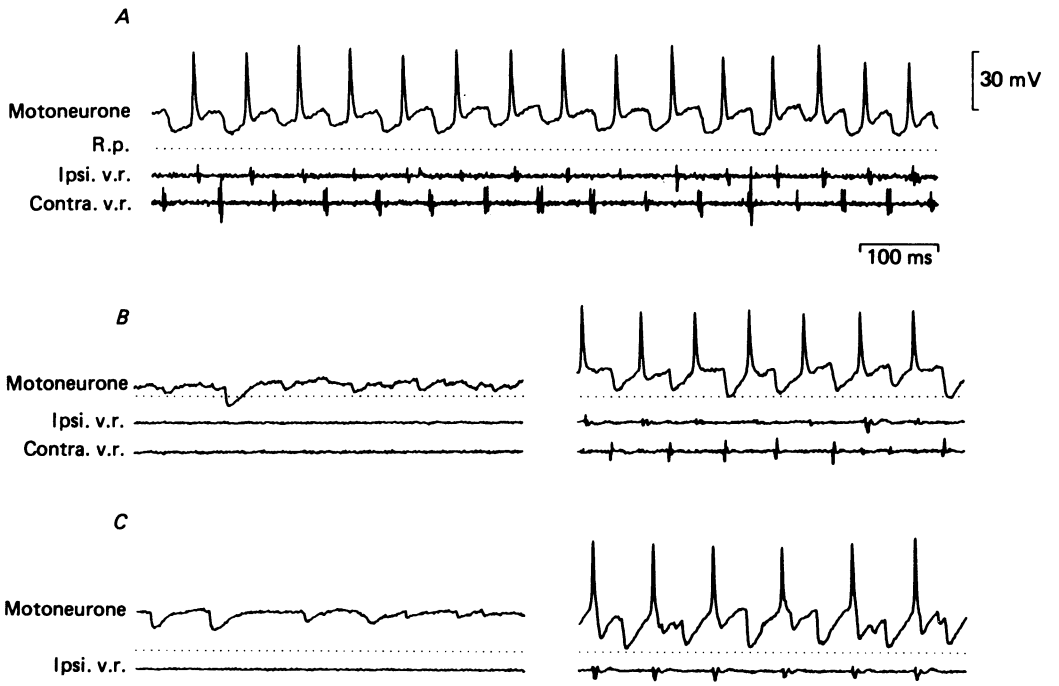

Fig. 3. Effect of NMDA and kainate. *A*, naturally evoked swimming in an intact animal (record kindly supplied by S. R. Soffe). Activity evoked in spinal embryos by *B*, 35 μM-NMDA and *C*, 6·5 μM-kainate applied in the bathing medium. The first half of *B* and *C* shows the phasic i.p.s.p.s which were evoked before fictive swimming started (shown in the second half of the Figure). Dots indicate the resting potential (r.p.) before swimming (*A*) and before agonist application (*B* and *C*). Note that in all cases the cells are tonically depolarized and that prominent hyperpolarizing potentials occur immediately after the spikes in (*C*). Abbreviations in this and subsequent Figures: ipsi. v.r., ipsilateral ventral root; contra. v.r., contralateral ventral root.

medium bathing embryos in which the myotomes overlying the spinal cord had been left intact resulted in sustained ventral root activity. This activity occurred 1–2 min after the agonist application, lasted 1–5 min and in twelve embryos had a pattern very similar to naturally evoked swimming in intact animals. When the myotomes overlying the spinal cord were removed to allow intracellular recordings from motoneurones, much lower concentrations of NMDA and kainate were required. NMDA at concentrations of 30–40 μM or kainate at 5–6·5 μM were sufficient to depolarize motoneurones and evoke a barrage of i.p.s.p.s which in six embryos was followed by sustained bursts of activity remarkably similar to naturally evoked swimming (see Figs. 2 and 3). At these concentrations, which were the only ones to

produce 'swimming-like' activity, NMDA and kainate caused a mean depolarization in motoneurones of 19·0 ± 1·80 (mean ± S.E. of mean) and 18·0 ± 2·00 mV, respectively, and decreased their input resistance by 23·0 ± 2·82 % and 24·0 ± 3·46 %, respectively (see Fig. 2 and Table 1). These values are very similar to those observed during the tonic excitation which occurs during naturally evoked swimming (Roberts &

TABLE 1. The effect of excitatory amino acid agonists on the motoneurone resting potential and input resistance in control, TTX and Mn^{2+} salines. Figures in parentheses are standard errors of the means. The number of animals n used for each agonist is also shown in parentheses

	Control saline		10^{-6} M-TTX		20 mM-Mn^{2+} + 0·5 mM-Ca^{2+}	
Agonist	Depolarization (mV)	% resistance change	Depolarization (mV)	% resistance change	Depolarization (mV)	% resistance change
30–40 μM-NMDA (n = 19)	18·8 (1·80)	−22·9 (2·82)	14·7 (1·90)	−17·6 (2·30)	–	–
5–6·5 μM-kainate (n = 26)	18·3 (2·00)	−24·2 (3·46)	14·5 (0·87)	−18·5 (3·12)	12·0 (0·65)	−13·5 (2·14)
5–8 μM-quisqualate (n = 18)	25·3 (1·84)	−35·5 (1·78)	25·5 (0·96)	−36·7 (3·20)	20·6 (1·73)	−29·5 (3·94)

Kahn, 1982; Soffe & Roberts, 1982*a*). Outside these critical concentration ranges NMDA and kainate depolarized motoneurones and increased their conductance. However, the only phasic activity observed under these conditions was the occurrence of sporadic i.p.s.p.s. The 'swimming-like' agonist-evoked activity, which was the only sort of spiking activity seen during NMDA and kainate application, consisted of one spike per cycle in phase with the ipsilateral ventral root spike (Fig. 3). The motoneurone spike had a prepotential which could be revealed by the injection of hyperpolarizing current (see Soffe & Roberts, 1982*b*). The spike alternated with a mid-cycle i.p.s.p. which was in phase with the contralateral ventral root spike (Fig. 2). The i.p.s.p. could be reversed by current injection (see Soffe & Roberts, 1982*b*). One slight difference between naturally evoked and agonist evoked 'swimming' was the presence of an often prominent hyperpolarization immediately after the spike in motoneurones (Fig 3*C*). While this is not a usual feature of motoneurone activity during naturally evoked swimming in intact animals it is sometimes seen (S. R. Soffe, personal communication).

The addition of 5–10 μM-quisqualate, while giving rise to activity lasting up to 1 min, never resulted in anything resembling the swimming pattern. In twelve embryos quisqualate at concentrations of 5 and 7 μM caused asynchronous unpatterned ventral root spikes, while at 10 μM it caused alternating longer bursts of ventral root spikes of uneven duration. During quisqualate application intracellular motoneurone recordings revealed a pattern of activity very different to that seen during NMDA and kainate application (Fig. 4). Spikes in motoneurones evoked during NMDA or kainate application were always either immediately preceded or followed by an

i.p.s.p.: quisqualate caused motoneurones to fire spikes unassociated with i.p.s.p.s (Fig. 4). Spikes and i.p.s.p.s evoked by NMDA and kainate were always in phase with the ipsilateral and contralateral ventral root spikes respectively. During quisqualate application there was only a very loose phase relation, if any, between ventral root spikes and phasic potentials in the motoneurone (Fig. 4). 5–8 μM-

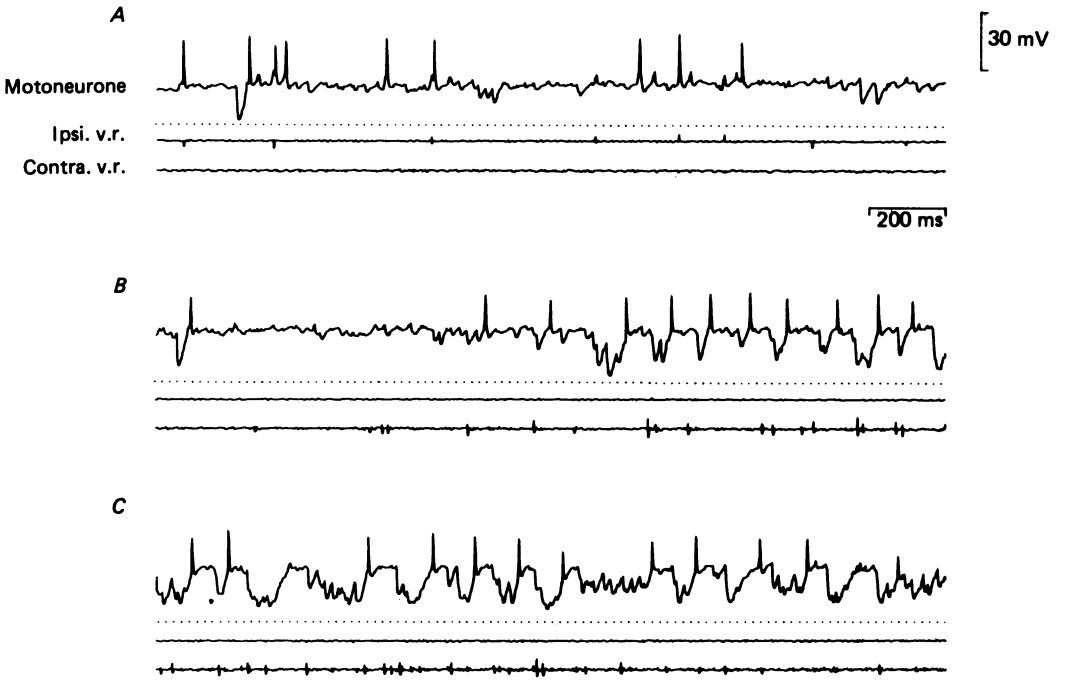

Fig. 4. The effect of 5 μM-quisqualate on a spinal embryo. *A*, *B* and *C* are three parts of the same experiment showing (in order) how the activity changed from sporadic spiking (*A*) to a slow alternation of excitatory and inhibitory potentials (*C*). Dotted line at resting potential before quisqualate application. Note how spikes in the ventral roots bear little or no consistent relationship to potentials in the motoneurone.

quisqualate caused a mean depolarization of $25{\cdot}0 \pm 1{\cdot}84$ mV and a mean resistance decrease of $36{\cdot}0 \pm 1{\cdot}78$ % in motoneurones. Both these values are significantly higher than those for NMDA and kainate (see Table 1). Finally when alternating excitatory and inhibitory activity was evoked in motoneurones by quisqualate it had a variable cycle period which was always longer than 100 ms. These observations suggest that the effect of quisqualate on motoneurones was qualitatively different to that of NMDA and kainate.

Agonist application in 20 mM-Mn^{2+} and TTX salines

The results in this section are from thirty-two motoneurones in thirty-two animals. In order to show that motoneurones possessed excitatory amino acid receptors, salines containing 20 mM-Mn^{2+} and 0·5 mM-Ca^{2+}, or 2×10^{-6} M-TTX were used to try and block synaptic transmission. Neither of these methods can be guaranteed to be

absolutely effective. Therefore electrical stimulation of the rostral spinal cord, which evokes synaptic potentials in motoneurones (Fig. 5*A*), was used as a criterion for the efficacy of these treatments. This stimulation can be assumed to activate Rohon-Beard cells, primary sensory neurones (Clarke, Hayes, Hunt & Roberts, 1984) and several

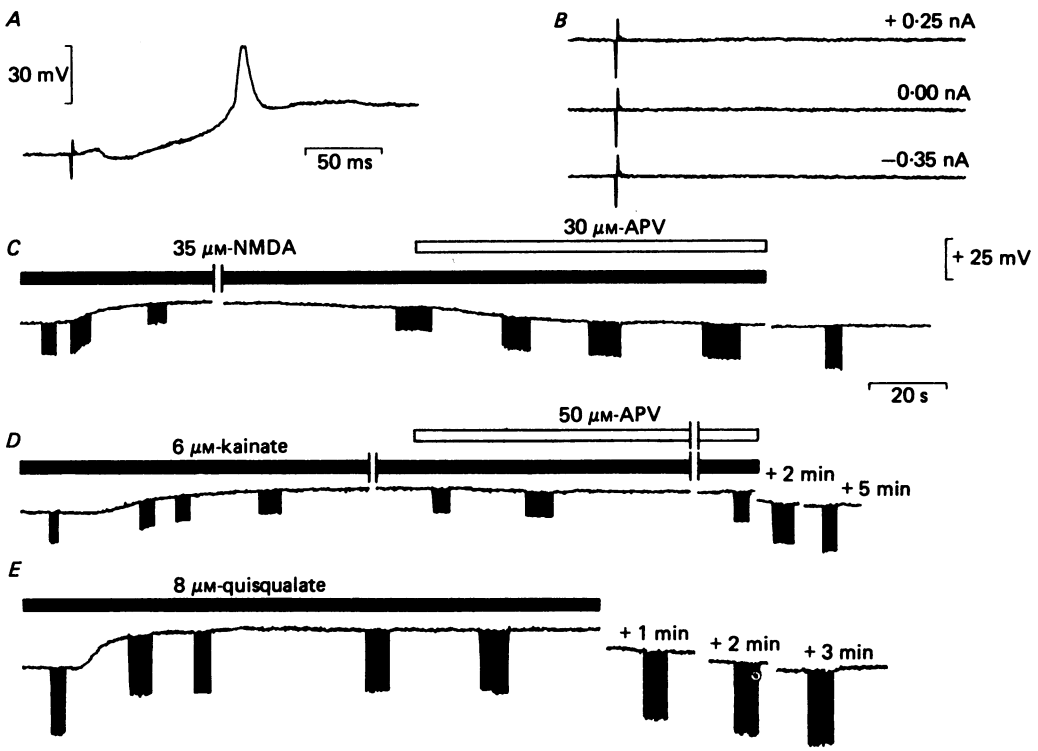

Fig. 5. *A* and *B*, the effect of saline containing 20 mM-Mn^{2+} and 0·5 mM-Ca^{2+} in blocking the response of motoneurones to rostral spinal cord stimulation. Before addition of the Mn^{2+} a short latency e.p.s.p. is followed by a long lasting depolarization (*A*). After the application of the Mn^{2+} no response to cord stimulation could be detected even during current injection (*B*). The effect of 35 μM-NMDA (*C*) and 6 μM-kainate (*D*) in 2×10^{-6} M-TTX saline and 8 μM-quisqualate (*E*) in saline containing 20 mM-Mn^{2+} and 0·5 mM-Ca^{2+} on motoneurone resting potential and input resistance (measured by constant hyperpolarizing current pulses of Fig. 1). *C* and *D* also show the effect of APV applied for 4 min in reducing the depolarization and conductance change evoked by NMDA and kainate respectively.

types of ascending and descending axons (Roberts & Clarke, 1982). Synaptic transmission was considered to be blocked when all spontaneous potentials had ceased and when electrical stimulation of the spinal cord failed to evoke any potentials in motoneurones (Fig. 5*B*). 2×10^{-6} M-TTX also rapidly abolished spikes evoked in motoneurones following intracellular current injection.

When these criteria had been met the agonist was added to the bathing medium. Very occasionally, even when the Mn^{2+} salines had been bathing the embryo for 15 min before agonist application, a few small phasic hyperpolarizing potentials

(presumably i.p.s.p.s) were seen superimposed upon the smooth agonist-evoked depolarization of the motoneurone. In TTX salines these i.p.s.p.s were seen more often and were more numerous. However, TTX and Mn^{2+} considerably reduced the number and frequency of i.p.s.p.s occurring in motoneurones (compare Figs. 2 and 5).

Both quisqualate and kainate were applied in TTX and Mn^{2+} salines. NMDA was applied in TTX salines only, because Mn^{2+} at submillimolar concentrations is an effective NMDA antagonist in the frog and rat spinal cords (Ault, Evans, Francis, Oakes & Watkins, 1980). NMDA and kainate caused a smooth depolarization in motoneurones when applied in the Mn^{2+} and/or TTX salines (Fig. 5*C* and *D*). The magnitudes of these depolarizations and their associated resistance changes were less than those evoked by these agonists in normal salines (see Table 1). However, only in the case of kainate applied in Mn^{2+} was this difference significant ($P < 0.05$). This may suggest that both NMDA and kainate also excite motoneurones through polysynaptic pathways.

Quisqualate application in TTX evoked a large depolarization, not significantly less than that evoked in control salines, superimposed on which were many phasic hyperpolarizing potentials. In Mn^{2+} salines quisqualate still caused a depolarization and conductance change (Fig. 5*E*) but there were almost no i.p.s.p.s. Under these conditions the depolarization and conductance changes were *not* significantly ($P > 0.05$) lower than those obtained in control or TTX salines (see Table 1).

The resistance of some i.p.s.p.s to TTX suggests the presence of a population of inhibitory cells within the spinal cord with TTX-resistant action potentials.

Simultaneous agonist and antagonist application

To show that excitatory amino acid receptors in *Xenopus* embryos were similar to those in other systems, salines containing both an excitatory amino acid agonist and antagonist were applied to spinal embryos. Combinations of NMDA, kainate or quisqualate with one of the three antagonists APV, DGG or PDA were added to the bathing medium, and each experiment repeated three times. Any resulting activity was monitored by ventral root recordings. In spinal embryos which had previously been shown to respond to skin stimulation with a brief flutter of ventral root spikes, a combination of 70 μM-NMDA and 50 μM-APV failed to evoke any ventral root activity. Similarly 10 μM-kainate in the presence of 1 mM-PDA or 0·5 mM-DGG failed to evoke any ventral root activity. However, 8 μM-quisqualate was able to evoke sustained bursts of ventral root spikes in the presence of 50 μM-APV, 2 mM-PDA or 0·5 mM-DGG, suggesting that these agents were ineffective as quisqualate antagonists.

As a final control the effects of APV on kainate- and NMDA-evoked depolarizations in motoneurones were examined by adding agonist and antagonist after agonist alone. 30 μM-APV quickly abolished the depolarization and conductance change previously evoked by 35 μM-NMDA (Fig. 5). However, 50 μM-APV had no significant effect on 6 μM-kainate-evoked depolarizations and conductance changes in motoneurones (Fig. 5). This last experiment was done in TTX salines to avoid the possibility of kainate activating NMDA receptors indirectly via a polysynaptic pathway.

The antagonists had no complicating agonist activity: when applied on their own

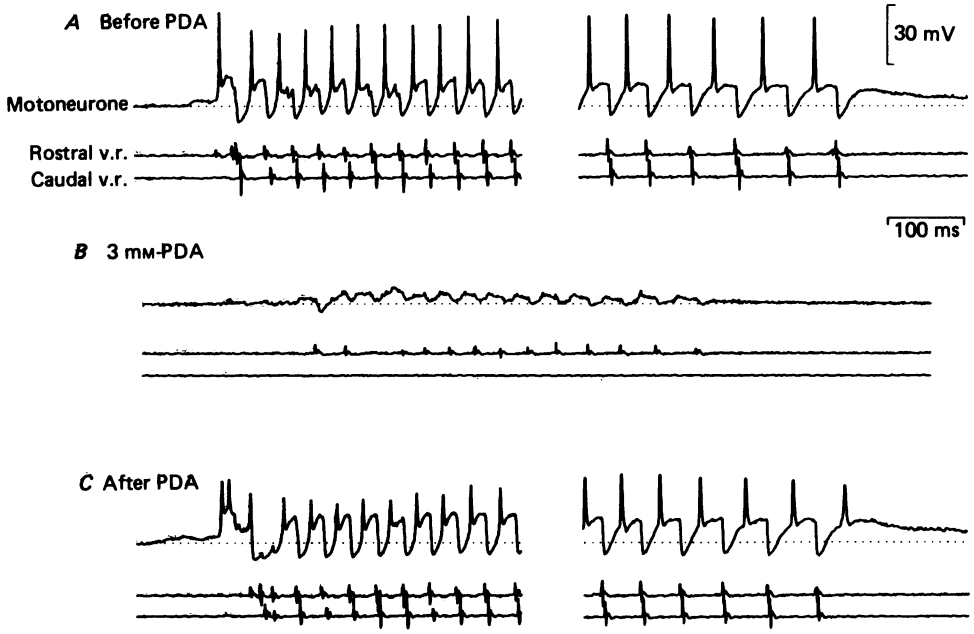

Fig. 6. The effect of 3 mM-PDA application to the spinal cord on swimming evoked by dimming the lights in a partitioned preparation. In *A* and *C* only the beginning and end of swimming episodes are shown. *A*, before PDA application, *B*, 3·5 min after PDA application and *C*, 4 min after PDA had been washed off. Resting potential during the swimming episodes is shown dotted.

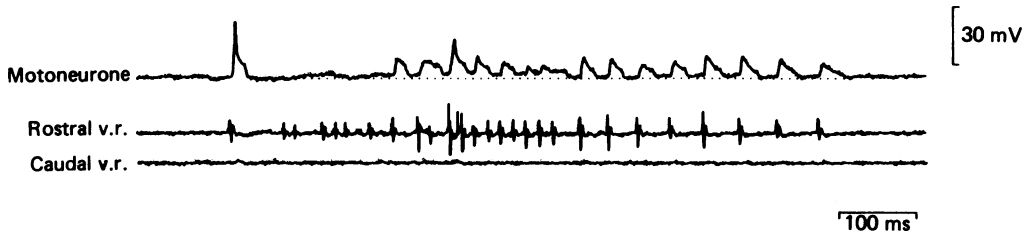

Fig. 7. Activity evoked in a partitioned preparation by dimming the lights 4 min after 3 mM-PDA application. The phasic e.p.s.p.s remaining in the virtual absence of tonic excitation are clearly visible.

(in particular PDA) they never depolarized motoneurones or changed their conductance.

There appear to be three distinct excitatory amino acid receptors in the *Xenopus* embryonic spinal cord which have pharmacological properties very similar to excitatory amino acid receptors in the adult frog and cat spinal cords (Watkins & Evans, 1981).

The partitioned preparation

The results described here are from thirty motoneurones in twenty-four animals and twenty-five experiments in twenty-five embryos with extracellular electrodes

only. The partitioned preparation, illustrated in Fig. 1, was devised to see whether excitatory amino acids are involved in mediating the tonic excitation of motoneurones that occurs during naturally evoked swimming. The head half of an embryo remained bathed in a control saline while the tail half was superfused with a saline containing an excitatory amino acid antagonist. This allowed natural stimulation, dimming the lights (Roberts, 1978) or occasionally electrical stimulation of the head skin (Roberts, 1980) to be used to turn on swimming. Ventral root recordings from the rostral half acted as controls to show that dimming the lights was an effective stimulus which evoked swimming.

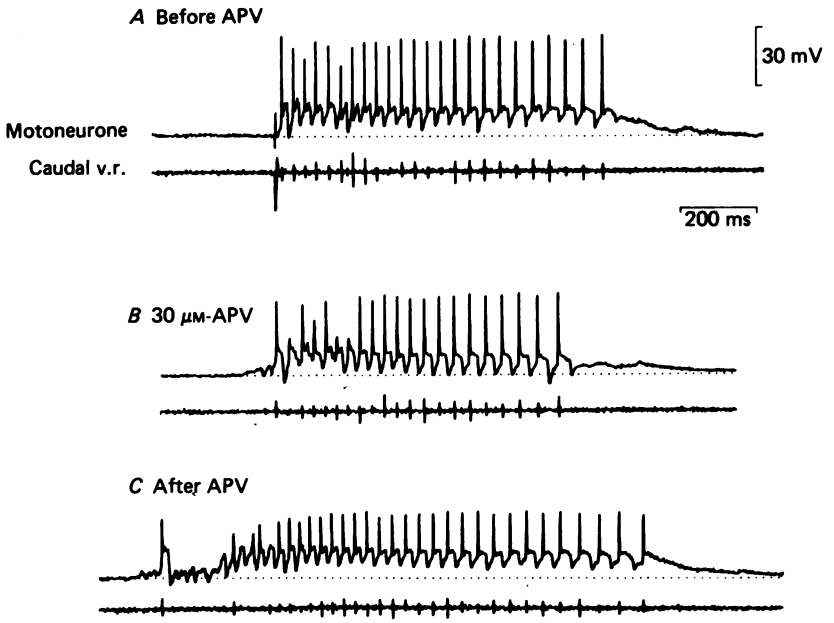

Fig. 8. The effect of 30 μM-APV on naturally evoked swimming in a partitioned preparation. *A*, before APV application, *B*, 8 min after APV application and *C*, 5 min after the APV was washed off. Dotted line indicates the resting potential. Note that the swimming episode in *A* was evoked by electrical stimulation of the head skin and in *B* and *C* by dimming the lights, and that the tonic excitation during activity in the caudal ventral root was *not* abolished by the APV.

Effect of antagonists on the tonic excitation

Two to three minutes after the application of 2–3 mM-PDA or 0·5 mM-DGG, dimming the lights could evoke ventral root activity only in the rostral half (Fig. 6). This activity was rhythmic and recognizable as swimming, but the swimming episode length (a variable phenomenon) and ventral root spike amplitude (a compound potential) were often reduced compared to their values before antagonist addition (Fig. 6). Intracellular recordings from motoneurones in the caudal half showed that the tonic excitation was much reduced, while some rhythmic phasic excitatory and inhibitory input remained (see Figs. 6 and 7). The phasic excitatory

and inhibitory potentials were reduced in amplitude by the antagonist application. During naturally evoked swimming in intact animals e.p.s.p.s and i.p.s.p.s vary in size (Soffe & Roberts, 1982*a*; S. R. Soffe, personal communication) and are probably compound potentials. The reduction in size of these potentials could therefore be due to a decrease in the number of components underlying them (see Discussion).

The application of APV in concentrations ranging from 30 to 200 μM only rarely abolished caudal ventral root activity (five out of twenty-one animals) and reduced the tonic excitation to a lesser extent than PDA or DGG (see Fig. 8).

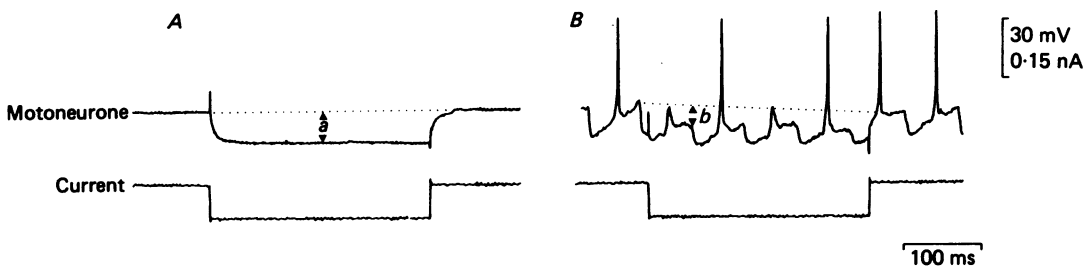

Fig. 9. The method by which the motoneurone input resistance changes which occur during swimming were measured. The amplitude of the potential produced by a hyperpolarizing current pulse was measured before swimming (*A*) and immediately before the mid-cycle i.p.s.p. during swimming (*B*). The percentage change in the cell input resistance ΔR was therefore $100(a-b)/a$.

Effect of antagonists on the associated resistance change

The tonic excitation of motoneurones during swimming has an associated resistance change which can be measured by passing constant hyperpolarizing current pulses into the cell before and during swimming (Roberts & Kahn, 1982; Soffe & Roberts, 1982*b*). The input resistance of the motoneurone was measured at mid-cycle (see Fig. 9), a time when the conductances due to spikes, phasic e.p.s.p.s and i.p.s.p.s should be minimal.

The i.p.s.p.s themselves do not affect the mid-cycle resistance of motoneurones (Soffe & Roberts, 1982*b*). If the resistance of motoneurones which do not receive a mid-cycle i.p.s.p. is measured immediately prior to a spike (a time when the conductance changes associated with spikes and phasic e.p.s.p.s are at their smallest) similar values to those measured at mid-cycle are obtained. Such cells are found contralateral and caudal to a hemisection of the rostral spinal cord (Soffe & Roberts, 1982*b*). Therefore, changes in the input resistance of motoneurones occurring at mid cycle can reasonably be taken as a measure of the resistance change associated with the tonic excitation. The size of this resistance change decreases throughout a swimming episode and is closely correlated with the cycle period (Roberts & Kahn, 1982). To allow comparisons between swimming episodes, measurements for a particular experiment were always made at a particular cycle period.

Both PDA and DGG abolished the mid-cycle resistance change within 2–3 min of their addition to the bathing medium (Fig. 10). APV reduced but did not abolish this resistance change (Fig. 11). Therefore, at least two receptors, one of which must be the NMDA receptor, appear to be involved in mediating the tonic excitation which

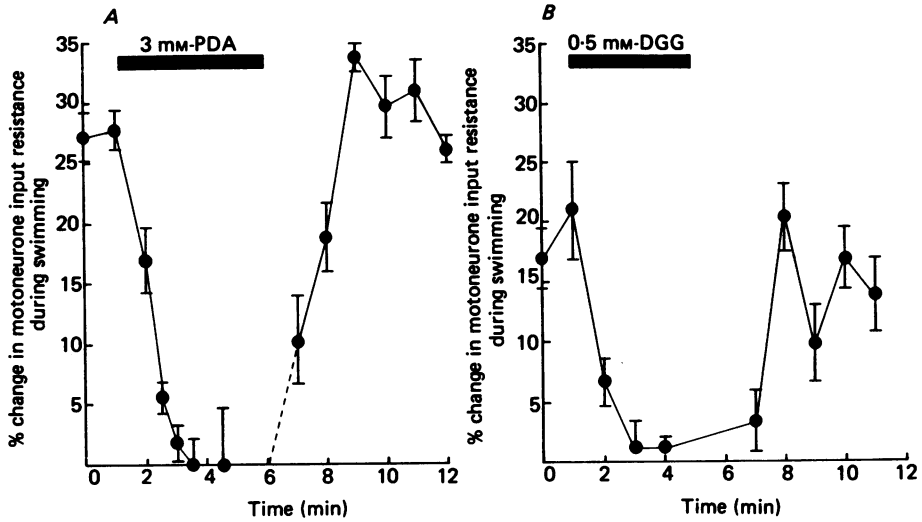

Fig. 10. The effect of *A*, 3 mM-PDA and *B*, 0·5 mM-DGG on the percentage change in motoneurone input resistance during swimming (ΔR).

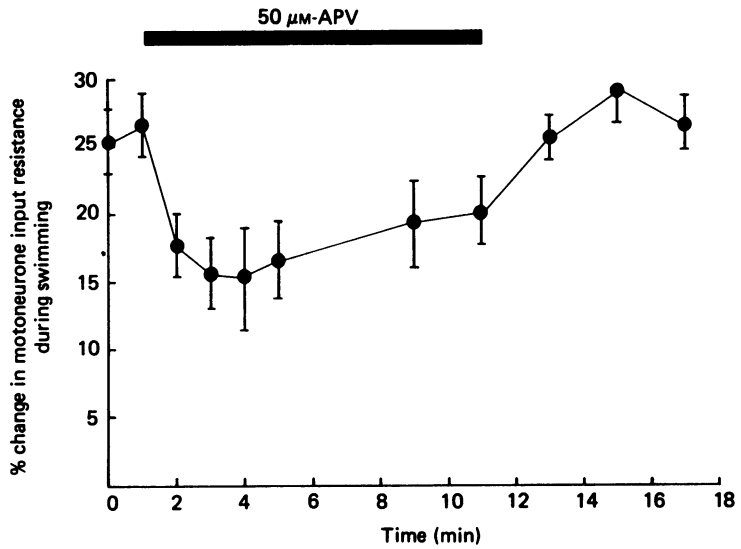

Fig. 11. The effect of 50 μM-APV on the change in motoneurone input resistance during swimming.

occurs during swimming. The activation of these receptors appears to be necessary for the generation of a patterned output from the spinal cord.

DISCUSSION

Receptor types and their location in the spinal cord

All three excitatory amino acid agonists will depolarize motoneurones, the effects of NMDA and kainate being similar while the effect of quisqualate is qualitatively

different. The actions of NMDA and kainate can be distinguished by APV which only antagonizes the effect of NMDA. Therefore, three types of excitatory amino acid receptor seem to be present in the *Xenopus* embryonic spinal cord. These receptors appear similar in their sensitivities to antagonists to those described elsewhere (Watkins & Evans, 1981). Unlike some other systems PDA did not have a partial agonist effect: when applied in the bathing medium it never caused a depolarization or change in the input resistance of motoneurones.

If NMDA receptors are present on motoneurones as the results with TTX salines suggest (see below), then activation of these receptors causes a conductance *increase*. In other systems, unlike the majority of other excitatory amino acid agonists, NMDA has been reported to cause a conductance *decrease*. Watkins & Evans (1981) put forward an explanation for this anomalous result, pointing out that in large neurones conductance changes occurring on distant dendrites due to NMDA application could be masked by other changes occurring nearer to the recording site in the cell soma. Since the motoneurones of *Xenopus* embryos have small somata (10–15 μm) and short dendrites (Roberts & Clarke, 1982) an electrode placed in the cell body may well detect conductance changes occurring in any part of the cell.

Crucial in deciding whether motoneurones possess excitatory amino acid receptors or not is the assumption that the TTX and Mn^{2+} blocked all or most of the synaptic transmission in the spinal cord. A negative assertion of this sort is impossible to prove. Neither treatment was entirely successful in blocking all inhibitory synapses (see Results). Since the spinal cord is small and easily permeable, TTX and Mn^{2+} salines can be assumed to block virtually all synapses unless there are mechanistic reasons for expecting otherwise, e.g. non-spiking cells, TTX resistant spikes or electrogenic synapses. Similar results were obtained with agonist application in TTX and Mn^{2+} salines. Since the mechanisms by which these treatments act to block synaptic transmission are independent this is evidence that virtually all synaptic transmission is blocked by TTX and Mn^{2+} salines. If this is the case, then all three excitatory amino acid receptors would seem to be present on motoneurones. In addition kainate and NMDA may also activate a polysynaptic pathway for motoneurone excitation.

Fictive swimming in spinal animals

The bath application of NMDA and kainate at particular concentrations to spinal animals can evoke a pattern of sustained motor output appropriate for swimming. These agonists also caused maintained depolarizations and changes in input resistance of motoneurones very similar to those associated with the tonic excitation which occurs during fictive swimming in intact animals. The application of NMDA and kainate in the bathing medium therefore appears to mimic the naturally occurring tonic excitation. NMDA can evoke fictive swimming in the lamprey (Grillner *et al.* 1981) where a similar explanation has been put forward. However, Poon (1980) found, also in the lamprey, that kainate application resulted only in unpatterned activity.

While quisqualate receptors are present in the *Xenopus* spinal cord they may not be involved in turning on or maintaining swimming since concentrations of PDA and DGG which blocked the tonic excitation were ineffective antagonists of quisqualate-evoked activity in spinal animals. Also the application of quisqualate resulted in a motor output which was inappropriate for swimming.

The experiments described have concentrated on motoneurones. However there are spinal cord interneurones which are rhythmically active during naturally evoked swimming which also receive tonic excitation (Clarke *et al.* 1983). When swimming was caused in spinal preparations by agonist application, motoneurones received on-cycle e.p.s.p.s and mid-cycle i.p.s.p.s. Therefore the premotor interneurones which generate this phasic patterned input also appear to be activated by NMDA and kainate.

Tonic excitation in swimming

The effects of the excitatory amino acid antagonists in the partitioned preparations provide evidence that excitatory amino acids are involved in mediating the tonic excitation of motoneurones during naturally evoked swimming. Since motoneurones possess all three excitatory amino acid receptors, the tonic excitation may be caused by direct release of the endogenous excitatory amino acid neurotransmitter onto synaptic sites on the motoneurone. Since PDA and DGG caused a greater reduction of the tonic excitation than APV, at least two excitatory amino acid receptors seem to be involved. The action of APV, and the effects of exogenous NMDA and kainate addition discussed earlier, imply that the tonic excitation is mediated via NMDA and kainate receptors.

Although the resistance change associated with the tonic excitation was completely abolished by excitatory amino acid antagonists some residual depolarization of motoneurones during swimming remained. Does this mean that another neurotransmitter is involved? Excitatory amino acid agonists are sufficient to evoke fictive swimming in spinal animals. Activation of excitatory amino acid receptors is necessary to allow a pattern generation. If another transmitter was involved it would be neither sufficient nor necessary for pattern generation. The residual depolarization could be explained in three ways which are consistent with excitatory amino acids mediating the whole of the tonic excitation. The antagonists may not have completely blocked the tonic excitation. The small depolarization of the motoneurone may have been caused by any remaining resistance change perhaps too small to have been measured. Alternatively, the depolarization could have been due to an accumulation of extracellular K^+ resulting from the activity of descending fibres. The resting potential of *Xenopus* Rohon-Beard cells is sensitive to the extracellular K^+ concentration (Spitzer, 1976). During swimming Rohon-Beard cells are normally inactive, but some may be depolarized by a few millivolts, due perhaps to raised extracellular K^+ levels (Clarke *et al.* 1984). The third possible explanation would be that the slight over-all depolarization was due to the summation of the remaining phasic e.p.s.p.s. These are known to extend over about 50–60 ms (Soffe & Roberts, 1982*b*) which is comparable to the cycle period at the beginning of a swimming episode. As the cycle period increased towards the end of an episode so the effects of phasic e.p.s.p. summation would be less.

The origin of phasic drive to motoneurones

Even when the tonic excitation had been largely abolished by excitatory amino acid antagonists, phasic excitatory and inhibitory input remained. This probably came, via descending axons, from the rostral spinal cord which was bathed in a

control saline. This portion of the central nervous system was still active and produced rhythmic motor output in the rostral ventral roots. The anatomy of *Xenopus* spinal cord interneurones, many of which have long descending axons, is consistent with this hypothesis (Roberts & Clarke, 1982). Soffe & Roberts (1982*b*) have presented independent evidence that phasic excitatory and inhibitory inputs to motoneurones originate, at least in part, from the more rostral parts of the spinal cord.

Both the phasic e.p.s.p.s and i.p.s.p.s were reduced in size during antagonist application. Since both are probably compound potentials, this size reduction could be explained as a progressive reduction in the number of components underlying the p.s.p., due perhaps to the slow diffusion of the antagonist along the length of the cord and beyond the partition. An alternative explanation for the e.p.s.p.s would be that they were excitatory amino acid dependent and that the reduction in their amplitude was a direct effect of the excitatory amino acid antagonist.

Being a tonic stimulus, the bath application of excitatory agonists is sufficient to cause spinal *Xenopus* embryos to generate a sustained motor pattern suitable for swimming. The action of these agonists seems to mimic the naturally occurring tonic excitation seen during swimming. If the tonic excitation is abolished selectively by excitatory amino acid antagonists the *Xenopus* spinal cord cannot generate a motor output; tonic excitation is therefore necessary for rhythmic motor output. The tonic excitation is mediated via a neurotransmitter acting at excitatory amino acid receptors.

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