SHORT COMMUNICATION

THE ACTION OF ACETYLCHOLINE ON THE LOCOMOTOR CENTRAL PATTERN GENERATOR FOR SWIMMING IN XENOPUS EMBRYOS

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Rhythmic locomotion in *Xenopus laevis* embryos is controlled by a central pattern generator in the spinal cord. This was demonstrated in experiments in which movements were blocked by tubocurarine or α-bungarotoxin (Kahn and Roberts, 1982a; Boothby and Roberts, 1988) and activity was recorded either extracellularly with suction electrodes on the ventral roots or intracellularly with microelectrodes from spinal neurones. Fictive swimming activity was evoked by skin stimulation, dimming of the illumination or could occur spontaneously, and its parameters were similar to those of actual swimming. This preparation, immobilised with neuromuscular blocking agents, has been very useful for the analysis of the cellular mechanisms underlying locomotor rhythm production (Roberts, 1990). The possibility remained that these antagonists also had central effects. To check this we have used an isolated nervous system preparation which enabled us to record fictive swimming activity in the absence of neuromuscular antagonists. This also allowed us to look at the effects of acetylcholine (ACh) and its antagonists on the central pattern generator for swimming locomotion.

Experiments were carried out using stage 37/38 *Xenopus laevis* embryos (Nieuwkoop and Faber, 1956). Animals were anaesthetised in 0.1% MS222 for 5 min and transferred to fresh saline (Soffe, 1990), where the central nervous system (CNS) was removed using etched tungsten pins. The isolated CNS consisted of about half to two-thirds of the spinal cord and the whole brain with pineal organ but without lateral eyes or olfactory bulbs. The CNS was then transferred to 5 ml of fresh saline in a Petri dish, where electrical activity (spinalogram) was recorded with a tapering polyethylene suction electrode applied to the caudal end of the spinal cord (Fig. 1). If a new Petri dish is used the CNS will stick to the bottom, facilitating intracellular recordings which were made with glass microelectrodes filled with 2 mol l⁻¹ potassium acetate, resistance 100–200 MΩ.

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In isolated nervous system preparations \((N=78)\) there were spontaneous bursts of spinalogram activity, lasting from several seconds to minutes, which occurred with very variable frequencies in different preparations (see Fig. 1A). Similar bursts of activity could be evoked by dimming the light (Fig. 1A) and these responses were abolished by removal of the pineal eye \((N=4)\). Isolated nervous systems remained active and responsive for many hours, in one case for 24 h.

To investigate the nature of this activity we made intracellular recordings from putative motor neurones \((N=9)\) in the ventral part of the spinal cord. Fig. 1B shows an example of such a recording together with the spinalogram activity. The intracellular activity was very similar to that recorded in motor neurones in the whole animal during fictive swimming (Soffe and Roberts, 1982). In the left part of the record each peak of activity in the spinalogram corresponds to a single spike in the intracellular record. The pattern of activity then changes so that there are two spinalogram peaks for each cycle of intracellular recording, one when the neurone fires its spike and the other when the neurone is inhibited. These two patterns of activity correspond to those described in the intact embryo (Kahn and Roberts, 1982b): 'synchrony' when both sides of the spinal cord fire together, and 'fictive swimming' when left and right sides alternate at frequencies from 10 to 25 Hz.
Acetylcholine and swimming

These two patterns cannot be distinguished in the spinalogram, where activity on both sides of the spinal cord is recorded.

We therefore used the isolated CNS and spinalogram to test the effects of ACh on the central pattern generator for swimming. Bath application of acetylcholine at 10^{-5} \text{mol}\,\text{l}^{-1} had a consistent effect on fictive swimming (N=10). If there was no spontaneous activity in control saline, application of ACh led to repeated strong bursts of spinalogram activity. When there was spontaneous activity in control saline, ACh led to an initial burst followed by a marked increase in the frequency of spontaneous bursts (Fig. 2A). In some cases, intracellular recordings from putative motor neurones showed that this activity was indistinguishable from fictive swimming.

To investigate which receptors were involved in these responses to ACh we have tested the effects of bath-applied antagonists: atropine sulphate (10^{-5} \text{mol}\,\text{l}^{-1}, Sigma), α-bungarotoxin (10^{-4} \text{mol}\,\text{l}^{-1}, Sigma) and d-tubocurarine (10^{-5} \text{mol}\,\text{l}^{-1}, Sigma). Application of atropine (N=5) reversibly blocked the effects of ACh.
Fig. 3. Effects of $10^{-5}\text{mol}\cdot\text{l}^{-1}$ acetylcholine (ACh) on six spinalised embryos in $\alpha$-bungarotoxin. Note the large increase in episode duration in ACh, followed by a smooth return to normal after about 15 min. This was observed in all embryos as well as for the grouped data. Episode durations for each embryo were expressed as a proportion of the control (normalised to 1).

(Fig. 2B). Atropine also considerably shortened both spontaneous and dimming-evoked bursts of fictive swimming ($N=6$, Fig. 2C) and in some cases dimming-evoked responses were abolished. In $\alpha$-bungarotoxin ($N=8$) and $d$-tubocurarine ($N=2$) the effects ACh application persisted. [Since Bixby and Spitzer (1984) have shown that $d$-tubocurarine antagonises GABA in this preparation its effects were not investigated further.]

As these results suggest that ACh effects are not antagonised by $\alpha$-bungarotoxin, it was possible to examine such effects on preparations where all visible movements had been blocked using this agent. In whole-embryo preparations ACh evoked a burst of activity, followed by an increased frequency of spontaneous swimming episodes, similar to that seen in isolated nervous systems ($N=7$, Fig. 2D). In spinalised embryos, where no spontaneous swimming was seen, ACh significantly increased ($P<0.01$) the length of swimming episodes evoked by electrical stimulation to the skin ($N=6$, Fig. 3).

Recordings from the isolated nervous system show that it is still capable of generating spontaneous fictive swimming activity and that, as in the intact animal (Foster and Roberts, 1982), this can be evoked via the pineal eye when the light is dimmed. This simple preparation, where extracellular spinalogram recording is easy and access of drugs is not impeded by surrounding tissue, should be valuable for pharmacological studies. As the pattern of activity during fictive swimming in the isolated nervous system is very similar to that in preparations immobilised in $\alpha$-bungarotoxin and $d$-tubocurarine we can draw two conclusions. First, these drugs do not appear to have strong effects on the central pattern generator, as indicated also by de-efferentation (Soffe, 1987). Second, the generation of the fictive
swimming pattern cannot depend on any mechanical feedback from the muscular system.

While nicotinic antagonists appear to have little effect, we have shown that ACh has a strong excitatory effect on the central pattern generator (CPG) and that this effect is blocked by the muscarinic antagonist atropine. The effect is twofold, increasing both the frequency of occurrence and the duration of spontaneous episodes of activity (Fig. 2). Since spinal animals show no spontaneous activity, the effect of ACh on frequency of occurrence of spontaneous episodes of activity is probably mediated by an action on neurones in the brain. However, the increased duration of swimming episodes is also clear in spinal animals, indicating that ACh is acting on the spinal CPG. The observation that atropine decreases the duration of spontaneous episodes (Fig. 2C) suggests that ACh is being released during fictive swimming but the neurones involved and their mode of action remain to be explored.

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References


