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SHORT COMMUNICATION

N-METHYL-D-ASPARTATE (NMDA) AND NON-NMDA TYPE GLUTAMATE RECEPTORS ARE PRESENT ON SQUID GIANT AXON SCHWANN CELLS

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The role of the amino acid glutamate in neuronal–glial signalling is becoming increasingly recognised in both vertebrate and invertebrate nervous systems (Barres, 1989). Although the ability of glutamate to depolarize cultured astrocytes has been known for some time (Bowman and Kimelberg, 1984; Kettenmann and Schachner, 1985), there has been a debate over whether this was due to a direct activation of glutamate receptors or resulted from an electrogenic glutamate uptake mechanism (Brew and Attwell, 1987; Cull-Candy *et al.* 1988). However, recent evidence using patch-clamping techniques suggests the presence of ionic currents activated by glutamate receptors in cultured astrocytes (Sontheimer *et al.* 1988; Usowicz *et al.* 1989). Responses to glutamate have also been obtained from glial cells in a number of intact preparations, including the adaxonal glia of the squid giant axon (Villegas, 1978) and the glia of the optic nerve of *Necturus maculosa* (Tang and Orkand, 1986). The responses of the former preparation (Lieberman *et al.* 1989) and of the neuropile glia of the leech segmental ganglion (Ballanyi *et al.* 1989) have been demonstrated to be due to glutamate receptor activation. In all the above studies the glutamate receptors present on glial cells have been demonstrated pharmacologically to belong to the non-NMDA subtype. Here we present evidence, for the first time in any glial preparation, that NMDA type glutamate receptors are present in the adaxonal glial or Schwann cells of the giant axon of the tropical squid *Sepioteuthis sepioidea* and that they mediate a slow depolarization of these cells. In addition, these Schwann cells also possess a second distinct population of glutamate receptors that mediate rapid signalling

Key words: NMDA receptor, quisqualate/kainate receptor, glutamate receptor, Schwann cell, squid, *Sepioteuthis sepioidea*.

responses and are pharmacologically similar to the non-NMDA glutamate receptors described in other glial preparations (Ballanyi *et al.* 1989; Lieberman *et al.* 1989; Sontheimer *et al.* 1988; Usowicz *et al.* 1989).

The effectiveness of L-glutamate pulses of different duration were assessed by successive measurements of the electrical potentials of a series of Schwann cells by brief impalements from inside the axon, as described previously (Villegas, 1972, 1973, 1975). Short pulses (1 min) of L-glutamate initiated a transient, dose-dependent hyperpolarization of the membrane of the Schwann cell (Fig. 1) with a threshold of between 2×10^{-10} and 5×10^{-10} mol l^{-1}. This response is mediated *via* a glutamate-induced release of acetylcholine from the Schwann cells; this feeds back onto nicotinic cholinergic receptors on the Schwann cells to promote the hyperpolarization (Villegas, 1972, 1973, 1975; Lieberman *et al.* 1989). Recent evidence suggests that this cholinergic response is potentiated by an endogenous peptide, similar to vasoactive intestinal peptide, which is probably co-released with acetylcholine from the Schwann cells (Evans and Villegas, 1988; Evans *et al.* 1986). However, prolonged pulses (10 min) of L-glutamate, more concentrated than 10^{-9} mol l^{-1}, gave rise to a biphasic response in which a slow depolarization of the Schwann cell membrane followed the initial hyperpolarization (Fig. 1).

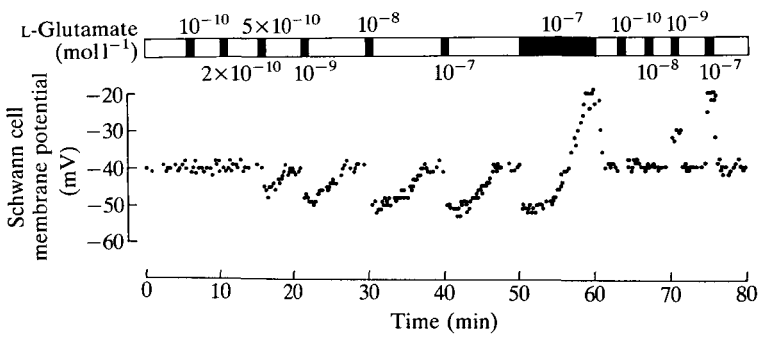

Fig. 1. Effect of 1 min pulses of various concentrations of L-glutamate and a 10 min pulse of 10^{-7} mol l^{-1} L-glutamate (solid bars) on the Schwann cell membrane potential. Each point represents the potential difference recorded in a different Schwann cell. Giant nerve fibres with a diameter of 300–400 μm were dissected in sea water from the hindmost stellar nerve of the squid *Sepioteuthis sepioidea*. Giant axons with their surrounding Schwann cell sheaths were then isolated and cleared of adhering bundles of small nerve fibres by dissection in artificial sea water (see below). Electrophysiological techniques were as described previously and involved the successive measurements of electrical potentials of a series of Schwann cells by brief impalements from inside the axon (Lieberman *et al.* 1989; Villegas, 1972, 1973, 1975). All experiments were carried out at room temperature (20–22 °C). A blind protocol was used in which the investigator sampling the Schwann cell membrane potentials did not know the identity of the test pulses being applied to the preparation. Drugs superfused over the surface of the preparation were dissolved in artificial sea water containing 442 mmol l^{-1} NaCl, 10 mmol l^{-1} KCl, 11 mmol l^{-1} $CaCl_2$, 45 mmol l^{-1} $MgCl_2$ and 10 mmol l^{-1} TrisCl buffer (pH 8.0). All the superfused solutions were continuously bubbled with a mixture of 95 % O_2 and 5 % CO_2. All drugs were obtained from the Sigma Chemical Company.

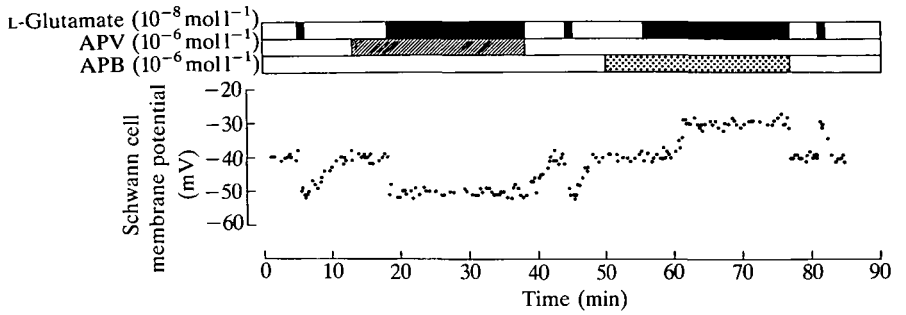

Fig. 2. The biphasic response of the Schwann cell membrane potential to a prolonged pulse of L-glutamate (10^{-8} mol l^{-1}) (solid bar) is converted into a rapidly induced sustained hyperpolarization in the presence of APV (10^{-6} mol l^{-1}) (hatched bar) and into a slowly developing sustained depolarization in the presence of APB (10^{-6} mol l^{-1}) (stippled bar). The hyperpolarizing response to a short pulse (1 min) of L-glutamate reverses polarity after the induction of the maintained depolarization. Each point represents the potential difference recorded in a different Schwann cell and the experiment has been repeated three times in this format.

Immediately after such a slow depolarization the response to a short pulse (1 min) of L-glutamate became a transient depolarization with a threshold of between 10^{-9} and 10^{-8} mol l^{-1}. Such transient depolarizations in response to the application of a series of short glutamate pulses become smaller with time and after 20–30 min they reverse to give hyperpolarizing responses (data not shown but see Fig. 3 below).

Pharmacological evidence indicates that the glutamate-induced slow depolarization of the Schwann cell membrane is mediated *via* NMDA type glutamate receptors. Fig. 2 shows that DL-2-amino-5-phosphonovaleric acid (APV or AP5) (10^{-6} mol l^{-1}), a selective NMDA receptor antagonist, blocked the induction of the slow depolarizing component of the response to a prolonged pulse of L-glutamate (10^{-8} mol l^{-1}). In the absence of the induction of a slow-depolarizing potential, the responses to short pulses (1 min) of L-glutamate were, like the initial control pulse, hyperpolarizing. Further, DL-2-amino-4-phosphonobutyric acid (APB or AP4) (10^{-6} mol l^{-1}) blocked the rapid hyperpolarizing response component of the glutamate response but left the slow depolarization intact (following a 5 min latency), after which the responses to a short pulse (1 min) of L-glutamate were again reversed. It should be noted that, although APB is thought to act as an agonist at a specific subtype of glutamate receptors in vertebrates, it has also been reported to act as a potent antagonist of glutamate receptors in several cephalopod muscles (Bone and Howarth, 1980; Florey *et al.* 1985; Lieberman *et al.* 1989). In addition, both APB and APV are active in the present preparation at much lower concentrations than in vertebrate preparations.

Further evidence of the differential nature of the receptors mediating the fast hyperpolarizing and slow depolarizing components of the glutamate response is provided by the responses of the preparation to quisqualate, kainate and NMDA.

Fig. 3 shows that short (1 min) pulses of all three derivatives induced hyperpolarizations of the Schwann cell membrane, with quisqualate (threshold between 10^{-8} and 10^{-7} mol l^{-1}) being more potent than kainate (threshold between 10^{-7} and 2×10^{-7} mol l^{-1}) and NMDA (threshold between 10^{-6} and 10^{-5} mol l^{-1}). Thus, the

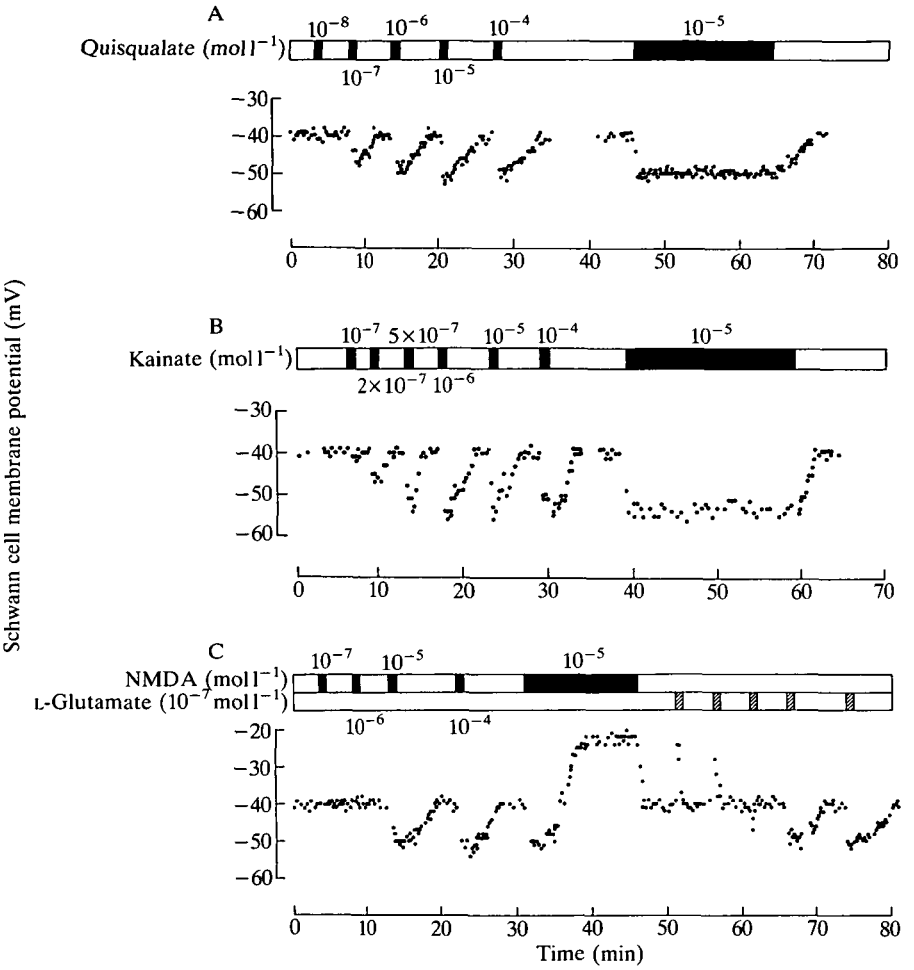

Fig. 3. Typical examples of the effects of 1 min pulses of various concentrations of quisqualate (A), kainate (B) and NMDA (C) (solid bars) on the Schwann cell membrane potential. (These experiments have been repeated three times in this format.) Prolonged pulses of 10^{-5} mol l^{-1} quisqualate (A) and kainate (B) produce only a rapidly activated hyperpolarization of the Schwann cell membrane. By contrast, a prolonged pulse of 10^{-5} mol l^{-1} NMDA (C) produces a biphasic hyperpolarization followed by a slow depolarization similar to that produced by L-glutamate. In C, repeated short pulses (1 min) of 10^{-7} mol l^{-1} L-glutamate (hatched bars) given after the induction of the slow depolarization initially give a rapid depolarizing response, but this is reduced with time and reverses after 15–20 min to give hyperpolarizing responses. Each point represents the potential difference recorded in a different Schwann cell.

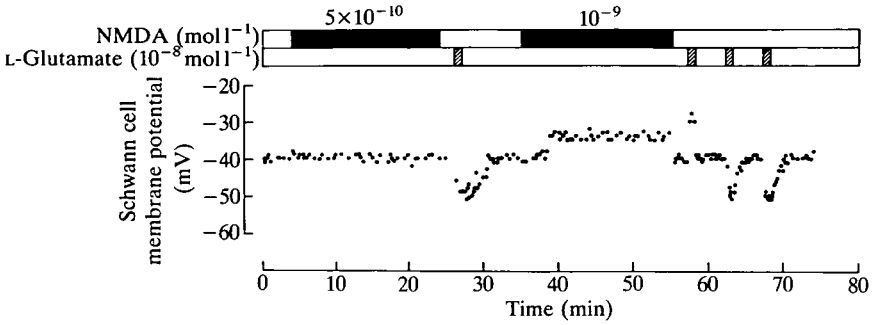

Fig. 4. Effect of prolonged pulses (20 min) of NMDA (solid bars) to show concentration threshold for the development of the slow depolarization of the Schwann cell membrane. At 5×10^{-10} mol l^{-1} NMDA no change in membrane potential is observed and a subsequent 1 min pulse of 10^{-8} mol l^{-1} L-glutamate (hatched bar) produces a rapid hyperpolarization. At 10^{-9} mol l^{-1} NMDA a slow depolarization is produced after a 5 min latency and the subsequent responses to a series of 1 min pulses of 10^{-8} mol l^{-1} L-glutamate (hatched bars) are transiently reversed in polarity. The prolonged pulse of 10^{-9} mol l^{-1} NMDA produces no initial hyperpolarization as this concentration is below the threshold for the activation of this response. Each point represents the potential difference recorded in a different Schwann cell and the experiment has been repeated twice in this format.

rank order of potency for the amino acid derivatives mediating this rapid hyperpolarizing response is glutamate>quisqualate>kainate>NMDA. However, neither quisqualate nor kainate applied as prolonged pulses (20 min), at concentrations up to 10^{-5} mol l^{-1}, was able to induce a slow depolarization of the Schwann cell membrane. In contrast, a prolonged pulse of NMDA (10^{-5} mol l^{-1}) did initiate a slow depolarization, after which the responses to a short pulse (1 min) of L-glutamate (10^{-7} mol l^{-1}) were again depolarizing but reversed their polarity after 15–20 min. The threshold for the NMDA-mediated slow depolarization occurred between 5×10^{-10} and 10^{-9} mol l^{-1} (Fig. 4) in contrast to the glutamate-mediated slow depolarization, which had a threshold between 10^{-9} and 10^{-8} mol l^{-1} (data not shown). The response to a short 1 min pulse of 10^{-8} mol l^{-1} L-glutamate was again transiently reversed in polarity after the induction of the slow depolarization by 10^{-9} mol l^{-1} NMDA. The slow depolarization in this instance was again induced after a 5 min latency. This latency appears to be common to all agonists that induce the slow depolarization.

The biphasic response of the Schwann cell membrane potential to a prolonged pulse of glutamate can also be converted to a sustained depolarization if the preparation is pretreated with α-bungarotoxin (Fig. 5), indicating that the transient hyperpolarization, but not the slow depolarization, is mediated by the activation of nicotinic cholinergic receptors, as outlined above. Further, if the calcium concentration of the superfusate is lowered from 11 to 0.11 mmol l^{-1} after such a glutamate-induced slow depolarization has been initiated, then the

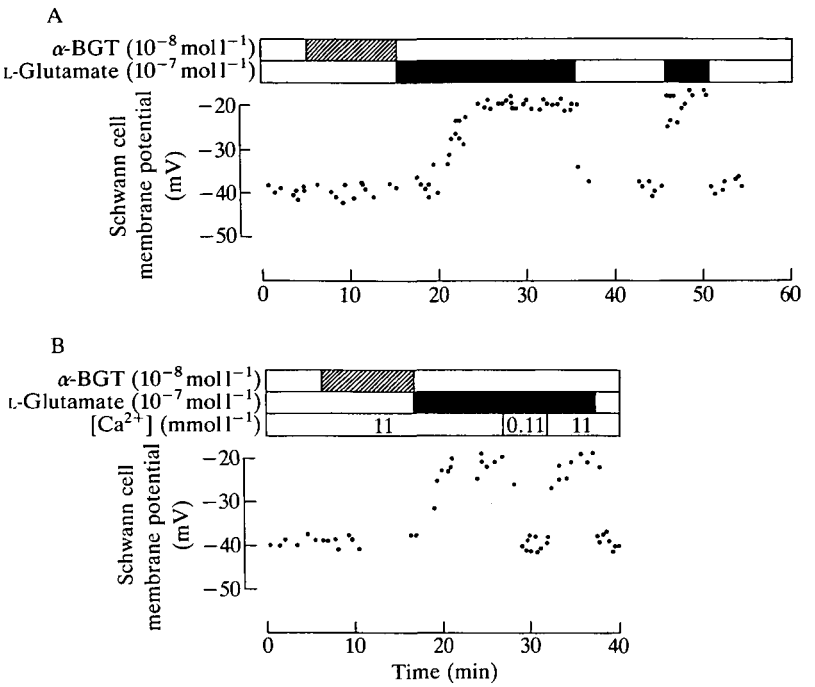

Fig. 5. Effect of 10^{-8} mol l^{-1} α-bungarotoxin (α-BGT, hatched bars) on the response of the Schwann cell membrane potential to prolonged pulses of 10^{-7} mol l^{-1} L-glutamate (solid bars). (A) α-Bungarotoxin blocks the rapid hyperpolarizing component of the glutamate response. (B) Reducing the calcium concentration (open bar) of the superfusate from 11 to 0.11 mmol l^{-1} rapidly abolishes the maintained depolarization, but it returns if the calcium level is returned to control values in the presence of glutamate. In the absence of α-bungarotoxin neither short (1 min) nor long pulses of L-glutamate produce any effect on Schwann cell membrane potential in the presence of low calcium concentrations (0.11 mmol l^{-1}) (data not shown). Each point represents the potential difference recorded in a different Schwann cell. The purified α-bungarotoxin was kindly supplied by Dr M. Raftery, Department of Chemistry, California Institute of Technology, USA.

sustained depolarization is abolished. However, it is rapidly re-established if the calcium concentration of the superfusate is returned to control levels, indicating that Ca^{2+} is needed to initiate and maintain this depolarization and that, as with NMDA receptors in neurones (Malenka *et al.* 1989), those present in the Schwann cell membrane also mediate an increase in calcium permeability. Thus, the slow depolarization may be due to a gradual accumulation of Ca^{2+} that changes the membrane properties of the Schwann cell.

This work shows that the adaxonal Schwann cells of the giant axon of the tropical squid *Sepioteuthis sepioidea* possess both NMDA and non-NMDA type glutamate receptors. The latter receptors can also be activated by glutamate released non-synaptically from the squid giant axon by stimulation at high frequency (Lieberman *et al.* 1989; P. D. Evans, V. Reale, R. M. Merzon and

J. Villegas, in preparation). Thus, they are probably involved in the complex signalling pathway whereby the membrane of the Schwann cell increases its permeability to potassium ions at times when the squid is likely to be using its giant-fibre-mediated escape response pathway (Villegas, 1981; Villegas *et al.* 1988). Under such circumstances the Schwann cells would be ideally suited to buffer any accumulation of potassium ions in the adaxonal space which might interfere with the conduction of impulses along the giant axons. At present, the physiological roles of the slow depolarization initiated by the NMDA receptors and the prolonged change in the physiological properties of the Schwann cell resulting from their activation remains unclear. They would, however, seem to be ideally suited to be activated after periods of prolonged glutamate release and indicate that the activation of NMDA receptors in a glial cell preparation can lead to long-term changes in responsiveness, as has been shown in a number of different neuronal systems (Davies *et al.* 1989; Kauer *et al.* 1988*a*,*b*; Malenka *et al.* 1989; Watkins and Collingridge, 1989).

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