Br. J. Pharmacol. (1990), **101**, 968–970

Philanthotoxin blocks quisqualate-, AMPA- and kainate-, but not NMDA-, induced excitation of rat brainstem neurones *in vivo*

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1 The effect of electrophoretic ejection of philanthotoxin (the polyamine toxin, from the Egyptian digger wasp) was tested on responses of brainstem and spinal neurones in the pentobarbitone-anaesthetized rat to excitatory amino acids.

2 Philanthotoxin caused a dose-dependent reduction of responses to quisqualate, α-amino-3-hydroxy-5-phenyl-4-isoxazolepropionate (AMPA) and kainate with little effect on those to N-methyl-D-aspartate (NMDA).

3 The time-course of this antagonist action was slow. In particular the rate of recovery was dependent on frequency of ejection of the agonist. This agonist-dependent recovery suggests that philanthotoxin has a channel blocking mode of action on mammalian central neurones.

Introduction

Transmission at the insect neuromuscular junction is mediated via postjunctional glutamate receptors of a type not dissimilar to those activated by quisqualate and α-amino-3-hydroxy-5-phenyl-4-isoxazolepropionate (AMPA), but quite distinct from N-methyl-D-aspartate (NMDA) receptors, in mammalian brain (Boden *et al.*, 1986). Venoms of orb-web spiders, containing low molecular weight polyamine-based toxins, paralyze their insect prey by a postsynaptic block at such neuromuscular junctions (see Jackson & Usherwood, 1988 for review). Philanthotoxin (PhTx), from the Egyptian digger wasp, *Philanthus triangulum*, is similar in structure and molecular weight to some of the above spider toxins, such as argiotoxin$_{636}$ (ATX) and Joro spider toxin (JSTX). Similarly, PhTx also causes neuromuscular paralysis when injected into honeybees on which the wasp preys (Eldefrawi *et al.*, 1988). Because of our interest in non-NMDA glutamate antagonists (Honore *et al.*, 1988) and because the actions of arthropod toxin have shown variable selectivity on mammalian glutamate receptors (Jackson & Usherwood, 1988), we decided to investigate the effects of PhTx on responses of rat central neurones *in vivo* to excitatory amino acids. A preliminary abstract has been published (Jones *et al.*, 1989).

Methods

Female Wistar rats (200–350 g), anaesthetized with 50 mg kg^{-1} sodium pentobarbitone i.p. were used in all experiments (see Honore *et al.*, 1988, for general methods). Briefly, the trachea, a major artery and vein were cannulated to maintain clear airways, record blood pressure and supplement anaesthesia, respectively. The rats were placed in a stereotaxic head frame and, after exposure of the dorsal medulla, recordings were made from single brainstem neurones with seven-barreled glass microelectrodes. Alternatively, the dorsal aspect of the spinal column was exposed and a partial lumbar laminectomy performed, allowing similar recordings to be made from single dorsal or ventral horn neurones in the lumbar cord. The microelectrode centre barrel, 4 M NaCl, was used to record action potentials; five of the six outer barrels were filled with solutions of: N-methyl-D-aspartate Na (NMDA, 200 mM, pH 8.1), quisqualate Na (5 mM in 200 mM NaCl, pH 7.8), α-amino-3-hydroxy-5-methyl-4-isoxazolepropionate Na (AMPA, 10 mM in 200 mM NaCl, pH 7.5), kainate Na (5 mM in 200 mM NaCl, pH 8.2), philanthotoxin (PhTx, 50 mM in 150 mM NaCl or 10 mM in 200 mM NaCl, pH 4.0), D-2-amino-5-phosphonovalerate Na (2-AP5, 25 mM in 175 mM NaCl, pH 7.6). The sixth barrel, 200 mM NaCl, was used for current balancing.

Action potentials from single brainstem neurones in the areas of the gracile, cuneate and trigeminal nuclei or lumbar spinal cord were monitored on an oscilloscope. Location of neurones was judged by the stereotaxic position of electrodes and from their responses to tactile stimuli of hindlimb, forelimb and oro-facial areas of skin. Firing rate of neurones was plotted continuously by a pen recorder and electrophoretic ejection of amino acids was adjusted to produce approximately equal and submaximal responses. After stable control responses had been obtained, PhTx or 2-AP5 was ejected and the size of the responses as a percentage of controls (mean ± s.d.) was taken to express the antagonist effect.

Results

Stable recordings were obtained from a total of 27 brainstem and 10 spinal neurones. Whether located in the gracile, cuneate or trigeminal nuclei, all brainstem neurones gave essentially similar results and so the data have been pooled. Although no differences with respect to pharmacology were observed between brainstem and spinal neurones in this study, data from these sets of neurones have not been pooled.

Administration of PhTx (−4 to +60 nA) selectively depressed quisqualate- and kainate-evoked firing of these neurones while having little or no effect on responses to NMDA. In the first experiments, very low ejecting currents of PhTx (50 mM in 150 mM NaCl) rapidly antagonized quisqualate responses. Subsequently, a 10 mM in 200 mM NaCl solution was preferred as this allowed more adequate control over the ejection of PhTx. Quisqualate and NMDA were tested on all 27 brainstem neurones. Responses to quisqualate were reduced to 25 ± 20% (mean ± s.d.) of control values, whereas those to NMDA showed an overall increase to 114 ± 40%. Responses to kainate, tested on 13 of the 27 neurones, were reduced to 36 ± 29%. On the same 13 neurones, quisqualate responses were reduced to 27 ± 21%. PhTx did not affect either the amplitude or the duration of the spike. At higher PhTx ejection currents, responses to NMDA were occasionally seen to diminish but, despite careful adjustments of the PhTx currents, there was no consistent difference in susceptibility of quisqualate and kainate responses to antagonism. Thus, in 29 tests of PhTx on 13 cells in which the antagonism of these two agonists by PhTx was compared, responses to quisqualate were reduced 20% more than those

Table 1 Selective effects of philanthotoxin (PhTx) on responses to quisqualate and kainate

Margin of difference	*Rank order* Quis > Kain	Kain > Quis
>10%	11	9
>20%	7	6
>30%	4	5

Frequency of occurrence of differences in sensitivity of quisqualate (Quis) and kainate (Kain) responses to PhTx, from 29 tests on 13 different neurones. On each neurone control responses to quisqualate and kainate were adjusted so as to give near equal responses before the administration of PhTx. The columns of numbers represent the times the responses to one agonist were reduced to a greater extent than the other. The rows show this analysis carried out at three separate levels of difference. Thus, in the middle row it can be seen that responses to quisqualate were reduced by 20% more than those to kainate on 7 occasions, whereas the reverse selectivity at this level was seen in 6 tests.

to kainate on 7 occasions and the reverse sensitivity was observed on 6 (see Table 1). 2-AP5 was tested on several of these neurones; its selective NMDA antagonist action was rapid in onset and recovered within 5 min of the end of its ejection. On 17 tests on a further 9 spinal neurones, PhTx reduced responses to AMPA by 87 ± 15%. On 5 of these cells, kainate responses were reduced by 96 ± 9% whilst responses to NMDA on all 9 cells were not affected significantly. Figure 1 illustrates the highly selective antagonist action of PhTx against non-NMDA responses on a spinal neurone. In several cases, after complete block of non-NMDA responses had been obtained, the PhTx ejecting current was increased several fold with no effect on responses to NMDA.

The full antagonist effect of PhTx at any given current was usually apparent within 5–10 min of the start of its ejection whereas, upon cessation of PhTx ejection, recovery was slow (Figure 1). In many cells with stable recording conditions full recovery often took up to 30 min. Because of this slow recovery and small changes in the cell-to-electrode tip relationship, full recovery from the effects of PhTx was only

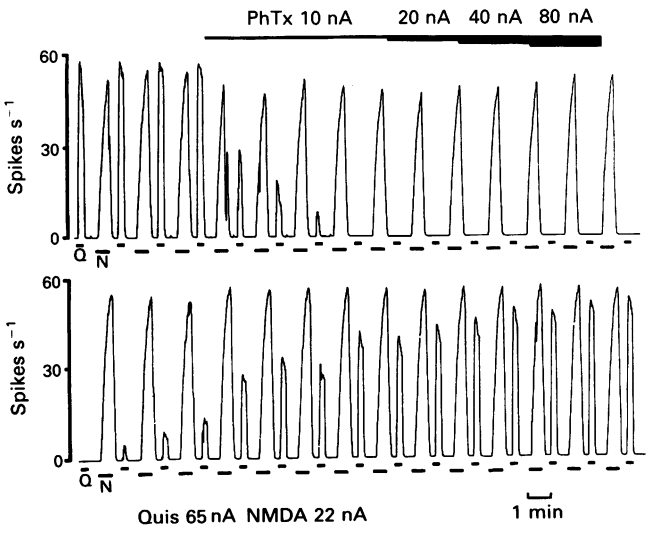

Figure 1 Ratemeter record from a single spinal neurone showing antagonism of quisqualate by philanthotoxin (PhTx) with no significant effect on responses to N-methyl-D-aspartate (NMDA). The uninterrupted upper record illustrates the gradual onset of action of PhTx (10 nA) to block the action of quisqualate (Quis, 65 nA). Further increases of ejecting current to 20, 40 and 80 nA were without effect on responses to NMDA (22 nA). The lower record shows the slow recovery from the effect of PhTx. There is a 15 min period of recording omitted between the upper and lower traces. Ordinate scale: firing rate in spikes s^{-1}.

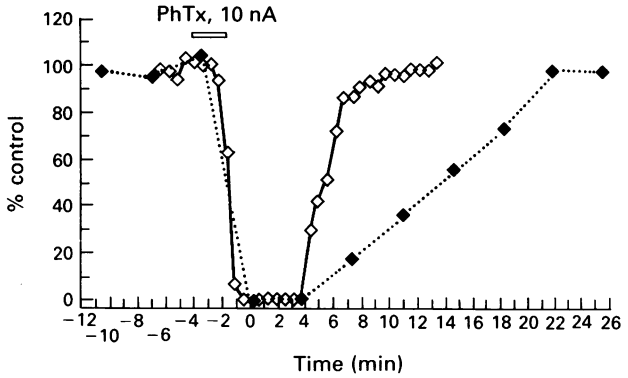

Figure 2 The difference in the time-course of recovery of responses to α-amino-3-hydroxy-5-phenyl-4-isoxazolepropionate (AMPA) 12 nA of a ventral horn spinal neurone, when the rate of agonist application is varied. In both cases AMPA responses were reduced to zero after 3–4 min application of philanthotoxin (PhTx). With fast application of AMPA (solid line; 20 s ejection, 15 s interval) the time taken for 50% recovery was approximately 4.5 min. With slower application (dotted line; 40 s ejection, 140 s interval), recovery to 50% of control took more than 13 min.

observed on about half the neurones tested. Nevertheless, the rate of recovery appeared to be dependent on the rate of agonist ejection, a result indicative of a channel blocking mode of action as suggested for its effects at the insect neuromuscular junction.

This was investigated in more detail by examining the rates of recovery with frequent or infrequent ejections of the agonist. On all cells tested, the rate of recovery from PhTx block of quisqualate or AMPA was slower for infrequent application (e.g. 20 s ejection at 3 min intervals) than for frequent application (e.g. 20 s every 20–40 s). An example of such a result, obtained from a ventral horn spinal neurone, is shown in Figure 2. This effect was not due to depolarization *per se* because frequent NMDA ejections did not hasten recovery of responses to AMPA or quisqualate.

Discussion

There are divergent views about the selectivity of the arthropod toxins for mammalian sub-types of glutamate receptors (Jackson & Usherwood, 1988), a situation which still exists in the more recent literature and to which must now be added the selectivity of PhTx toward non-NMDA subtypes of glutamate receptor observed here.

On the one hand, there is convincing evidence that they act as NMDA antagonists. Thus, the binding studies on rat brain membranes of Mena *et al.* (1989), the single cell voltage-clamp experiments (Kemp *et al.*, 1988) and whole animal experiments of Seymour & Mena (1989) all suggest that such toxins are NMDA blockers. PhTx has also been shown both to inhibit non-competitively NMDA-sensitive [^{3}H]-MK-801 binding to rat brain membranes and to reduce NMDA responses 10 times more effectively than non-NMDA responses in *Xenopus* oocytes (Ragsdale *et al.*, 1989). Furthermore, in support of an NMDA blocking action, PhTx reduces polysynaptic reflexes to a greater extent than monosynaptic reflexes in hemisected spinal cords of neonatal rats (N.A. Anis, unpublished observations).

On the other hand, other recent data provide contradictory evidence. Thus in electrophysiological studies on rat hippocampal neurones, Ashe *et al.* (1989) and Saito *et al.* (1989) demonstrated that ATX *in vitro* and JSTX *in vivo* respectively blocked glutamate-, but not aspartate-induced depolarizations of CA1 pyramidal cells. Ashe *et al.* (1989) also showed that ATX reduced Schaffer collateral-evoked field potentials, responses which are known to be mediated by non-NMDA receptors. All these effects were reversible on washing. The

present results with PhTx are in agreement with these latter studies.

In view of the structural similarity between PhTx, ATX and JSTX, we find it interesting to speculate upon whether small differences in structure may confer different selectivity for NMDA and non-NMDA receptor/channel complexes, or whether small differences in experimental protocol such as the extracellular environment may explain the apparent contradictory findings. It may be more instructive to compare several toxins on a given preparation.

With respect to the mode of action, our present results cannot provide a definitive answer, but the agonist-dependent nature of the recovery suggests that PhTx blocks and becomes trapped within the channels coupled to non-NMDA receptors. The failure of PhTx to distinguish between quisqualate and kainate is reminiscent of our results with the quinoxalinediones on spinal neurones, and supports our notion that quisqualate and kainate depolarize such central neurones by acting at similar or even common receptor-channel complexes (Honore *et al.*, 1988).

In conclusion, such toxins are potentially useful tools for investigating the pharmacology and physiology of glutamate receptor-channel complexes in the mammalian CNS and may have an important role to play in the design of useful therapeutic compounds.

This work was supported by Wellcome Trust grant 03057/Z/89 to D.L. and by NIH grants ES04977, ES02594 and AI 10187 respectively to Drs M. Eldefrawi, A.T. Eldefrawi and K. Nakanishi, whose help we also gratefully acknowledge.

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(Received May 21, 1990
Accepted July 25, 1990)