SYNAPTIC TRANSMISSION IN THE SIXTH GANGLION OF THE COCKROACH: ACTION OF 4-AMINOPYRIDINE

BY B. HUE, M. PELHATE, J. J. CALLEC* AND J. CHANELET
Laboratoire de Physiologie, Faculté de Médecine, 49000 Angers, France

(Received 12 April 1976)

SUMMARY

1. Study was made of the action of 4-aminopyridine (5 x 10^{-5} M) on synaptic transmission in the last abdominal ganglion of Periplaneta americana. The ‘oil-gap’ technique was used to record postsynaptic events in a single giant axon.

2. 4-AP quickly increased the ‘background’ of postsynaptic activity, which consisted of ‘spontaneous’ unitary EPSPs and IPSPs. Postsynaptic spikes were also propagated.

3. Both evoked EPSPs (stimulation of cereal nerve XI) and evoked IPSPs (stimulation of cereal nerve X) were greatly increased in amplitude although their duration (half-time) was unaltered.

4. 4-AP triggered presynaptic action potentials in the cereal nerves (recorded with external electrodes). These ‘antidromic’ potentials appeared singly or sometimes repetitively, especially after electrical stimulation of the cereal nerves. They were often in monosynaptic correlation with unitary EPSPs.

5. Neither the resting potential nor the postsynaptic membrane resistance was modified.

6. There were no changes in the equilibrium potentials of the ions involved in postsynaptic events.

7. The results may be essentially explained by an increase in transmitter release after 4-AP treatment, which may be partly the result of a rise in presynaptic terminal excitability, and partly the result of a lengthening of the presynaptic action potentials.

INTRODUCTION

4-aminopyridine (4-AP) has a convulsant action in vertebrates (Chanelet & Lemeignan, 1969; Lemeignan, 1970, 1971, 1972, 1973; Lemeignan, Chanelet & Saade, 1969; Saade, Chanelet & Lonchampt, 1971a, b), and a similar action in an insect, Periplaneta americana (Pelhate, Hue & Chanelet, 1972). Such action may be tentatively explained as being due to the effect of 4-AP upon either axons or synapses.

In axons, 4-AP produces a rather specific blockage of K+ channels (Pelhate & Pichon, 1974; Pelhate et al. 1974b, 1975, 1976; Meves & Pichon, 1975; Llinás, Walton & Bohr, 1976; Yeh et al. 1976). Action at a neuromuscular junction has

been shown by Molgo, Lemeignan & Lechat (1975), in the frog, to involve an increase in EPP amplitude and mean quantal content without modifying the spontaneous transmitter release. This may explain the 'curare antagonism' of Lemeignan & Lechat (1967) and Sobek et al. (1968).

Indirect evidence for action at neuromuscular junctions includes the observation that 4-AP potentiates the maximal contractions of the isolated phrenic nerve-diaphragm preparation of the rat (Fastier & McDowall, 1958; Kapff, 1959).

In this paper we present evidence that, in addition to affecting axons and neuromuscular junctions, 4-AP can affect central synapses, in the sixth ganglion of the ventral nerve cord of *P. americana*. Preliminary investigations of the effect of 4-AP upon this ganglion have already been published (Hue et al. 1975a, b). The sixth (terminal) ganglion contains synapses between afferent fibres of cercal nerves and giant neurones of the nerve cord. The technique that we have employed has allowed us to investigate the effect of 4-AP upon both presynaptic and postsynaptic activity.

**MATERIALS AND METHODS**

Details of the electrophysiological procedure have been previously described (Pichon & Callec, 1970; Callec, 1972). Essentially, the posterior end of the ventral nerve cord of the cockroach *Periplaneta americana*, attached to a cercus by cercal nerves X and XI (terminology of Roeder, Tozian & Weiant, 1960), was mounted in a Perspex chamber, after a dissection which left giant axon II (Harris & Smyth, 1971) as the connexion between the 5th and 6th ganglia (Fig. 1). Continuous recording of postsynaptic potentials, and of postsynaptic membrane potential and action potentials, was made from the giant neurone by employing an 'oil-gap'. The postsynaptic membrane potential could be shifted by the inclusion of a Wheatstone bridge in the recording circuit. Action potentials in the cercal nerves were triggered by stimulation of cercal mechanoreceptors and by electrical stimulation of the nerves through silver electrodes. With appropriate positioning of these electrodes, nerves X and XI could be stimulated independently. This allowed stimulation of either predominantly excitatory or inhibitory fibres - it seems that most of the afferent excitatory fibres are carried in nerve XI (Callec & Boistel, 1971; Callec, 1974), whereas cercal nerve X has an inhibitory influence (Callec, 1972, 1974). Recordings from the nerves were made with Ag/AgCl electrodes (Fig. 1).

The sixth ganglion was perfused with a Ringer containing 210 mM-NaCl, 3.1 mM-KCl, 5.4 mM-CaCl₂, 2 mM-NaHCO₃. 4-AP was added to the Ringer to give a final concentration of $5 \times 10^{-5}$ M.

All experiments were carried out at room temperature (18°C).

**RESULTS**

1. **Resting potential**

At 4-AP concentrations higher than $5 \times 10^{-5}$ M, the postsynaptic membrane is depolarized, following a characteristic sigmoid dose-response curve (Hue et al. 1975b). To study the action of 4-AP on postsynaptic potentials without interference with an eventual depolarization, the present experiments employed a concentration of $5 \times 10^{-5}$ M. A rough estimate of resting potential was obtained by depolarization,
Action of 4-aminopyridine in the cockroach

2. Unitary postsynaptic potentials

The cercal mechanoreceptors produced a ‘background’ of action potentials in the cerical nerves which induced unitary postsynaptic potentials (EPSPs and IPSPs) in the giant axon (Fig. 2A). With a 4-AP concentration of $5 \times 10^{-6}$ M, this activity greatly increased in frequency and amplitude (Fig. 2A2) but not duration, as indicated by the half-time for decay (Fig. 2B). EPSP summation could reach the threshold level which triggered propagated action potentials in the giant axon. The amplitude of IPSPs was increased less than that of EPSPs (which might be expected when IPSP equilibrium potential is closer to the resting potential than EPSP equilibrium potential – see below).

The increase in frequency of postsynaptic potentials indicates that there was increased activity of presynaptic terminals. Some action potentials recorded in the cerical nerves appeared to be ‘antidromic’ potentials generated by this activity, since such action potentials could precede EPSPs with a one-to-one relationship, as has been shown in preceding papers (Hue et al. 1975a, b). The mean delay was estimated at 0.7 ms, which is very close to the delay between an orthodromic cerical action potential and its correlated unitary EPSP (Fig. 2C and Callec, 1972, 1974). This is indirect evidence that there is a monosynaptic connexion between excitatory cerical afferents and the giant axon, as is indicated by recent data (Callec, 1974). A one-to-one relationship between the ‘antidromic’ action potentials and unitary IPSPs were never observed, which is in strong agreement with the assumed bi- or polysynaptic nature of the inhibitory pathway (Callec, 1972, 1974).

It may be objected that the so-called ‘antidromic’ potentials were conducted through efferent neurones. To test this, 4-AP was applied after inducing degeneration with isotonic KCl, of the cut end of the giant axon. This estimate has been used to evaluate equilibrium potentials (Figs. 6 and 7).
Fig. 2. Effects of 4-AP (5 \times 10^{-4} M) on postsynaptic background activity composed of unitary EPSPs and IPSPs. The cerci and cercal nerves XI and X were intact. (A) Ri: in normal Ringer; 4-AP: 10 min after 4-AP application. Note that EPSPs and IPSPs were numerous and that action potentials were triggered. (B) The action of 4-AP on amplitude and duration of unitary EPSPs and IPSPs. (C) Examples of EPSPs triggered by orthodromic (under Ringer-Ri) or 'antidromic' (under 4-AP) action potentials.

of the cercal fibres (by section of the cercal nerves near the cerci). 'Antidromic' potentials were never observed in such experiments.

3. Evoked monosynaptic EPSP

If sufficient electrical stimulation was applied to cercal nerve XI, there was summation of unitary EPSPs to give a postsynaptic depolarization—the evoked
Fig. 3. Action of 4-AP on evoked EPSP amplitude in the sixth ganglion, the cercal nerves and cerci being severed (A) or intact (B). (A1) Superimposed recordings (subthreshold stimulation). (a) In Ringer. (b) and (c) After perfusion with 4-AP for 2 and 4 min respectively. (A2) The effect of 4-AP on the relationship between EPSP amplitude and duration (several experiments). (B) Action of 4-AP on the subthreshold evoked EPSP. In B1, duration as well as amplitude of the EPSP has greatly increased over that in B1, but note that the electrotonic influence of the presynaptic potential remained unchanged. In B4, the intensity of stimulation has been lowered (about 25%). The presynaptic potential is not seen but the long depolarization is still able to induce propagated action potentials. At the same time, cercal nerve XI is invaded by a strong 'antidromic' volley. Ri = Ringer.

EPSP. The evoked EPSP could, upon reaching a threshold value, trigger a propagated action potential in a giant axon. In the presence of 4-AP, the subthreshold EPSP underwent a great increase in amplitude (Fig. 3A1) in less than 10 min. The electrotonically transmitted presynaptic potential was not altered (see Fig. 3B1-2), suggesting that, at this concentration, 4-AP was acting only at the synaptic level, i.e. there was no rise in cercal fibre recruitment. EPSP duration, as indicated by the half-time for decay, was not affected by 4-AP (Fig. 3A2).

During the above experiments, cercal nerves XI were cut, severing their connexion with the cerci, to prevent background activity interfering with the evoked EPSP. If the cercal nerves were left intact, the duration of the subthreshold EPSP appeared
522 B. Hue and Others

Fig. 4. Action of 4-AP on evoked IPSP in the sixth ganglion of the cockroach. The IPSP was quickly increased in amplitude but not significantly changed in duration (B). A large depolarizing component was often seen following the IPSP, as in A. Antidromic action potentials were often seen at the same time as this depolarizing component, as shown in the lower trace. *Ri*: Ringer.

to increase. This may be explained partly by a secondary effect through presynaptic terminals. The rather strong electrical stimulation of the cercal nerves was accompanied, in the presence of 4-AP, by an increase in terminal excitability which was represented by the appearance of ‘antidromic’ potentials, in correlation with postsynaptic potentials (Fig. 3B–4). This effect was less evident if the cercal nerves were severed. It is possible that background mechanoreceptor activity maintains the presynaptic terminals in an excitatory state which approaches the threshold level, allowing repetitive activity to be more easily induced (see Pelhate et al. 1972, 1976).

4. Evoked polysynaptic IPSP

In the same way that sufficient stimulation of nerve XI could produce an evoked EPSP by summation of the unitary EPSPs, sufficient stimulation of nerve X produced an evoked IPSP by the summation of numerous IPSPs. The sensory receptors which originate the inhibitory pathway are still unknown (Callec, 1972, 1974).

In the presence of 4-AP, the evoked IPSP is, like the evoked EPSP, increased in amplitude, whereas the duration remains relatively constant, as measured from half-time for decay (Fig. 4). A depolarization is often seen to succeed the IPSP. ‘Antidromic’ potentials sometimes occur during this EPSP, but not during the evoked IPSP.

It should be noted that 4-AP acts both on the frequency and the amplitude of the
postsynaptic responses. The frequency increase gives strong support to the view that 4-AP has a presynaptic action, most probably on the cercal fibre terminals. The amplitude increase, on the other hand, could be due to presynaptic mechanisms (i.e. increase in transmitter release) or to postsynaptic events affecting membrane electrical characteristics or ionic characteristics. To investigate the involvement of such postsynaptic events, study was made of membrane resistance and equilibrium potentials.

5. Postsynaptic membrane resistance

Fig. 5 shows that $5 \times 10^{-6}\text{M}$ 4-AP apparently results in little change in postsynaptic membrane resistance, as measured with hyperpolarizing current pulses, so such change in resistance is not the explanation of the significant increase in amplitude
of EPSPs or IPSPs (Figs. 3 and 4). When measuring resistance with depolarizing pulses, the 4-AP response was slightly increased and there was some repetitive activity. This may be associated with the effect of 4-AP upon the axonal part of cockroach giant neurones to decrease delayed rectification (Pelhate, Hue & Chanelet, 1974a).

6. **Equilibrium postsynaptic potentials**

**EPSP equilibrium potential** ($E_{EPSP}$).

The 'oil-gap' technique does not afford a direct evaluation of $E_{EPSP}$ in the cockroach sixth ganglion, although it can be estimated by extrapolation of a straight line between EPSP amplitude and membrane potential (Callec, 1972, 1974). However, where there is such a straight-line relationship (i.e. with hyperpolarizing pulses), comparison can be made between slopes in the presence and in the absence of 4-AP. 4-AP did not affect slopes in such experiments (Fig. 6), so we may assume that it does not alter equilibrium potentials for those ions involved in the generation of the EPSP, namely Na$^+$ and probably K$^+$ (Pitman & Kerkut, 1970; Callec, 1972, 1974).

**IPSP equilibrium potential** ($E_{IPSP}$)

The IPSP may be inverted at membrane potentials around $-80$ mV (Fig. 7). As with the EPSP, there was no indication of an alteration of equilibrium potential by 4-AP. The ions involved are believed to be Cl$^-$ and most likely K$^+$ (Pitman & Kerkut, 1970; Callec, 1972, 1974).
**DISCUSSION**

Our results show a significant effect of 4-AP at the synaptic level. Several sites of action for 4-AP may be proposed. At the presynaptic level, it may increase transmitter synthesis or release, perhaps at one particular stage of these processes. At both presynaptic and postsynaptic levels, it may counteract cholinesterase when acetylcholine is involved. Finally, at the postsynaptic membrane, it might modify electrical characteristics, or increase the sensitivity of transmitter receptors.

Some of the above possibilities are unlikely. Although acetylcholine appears involved as an excitatory transmitter in this ganglion (Callec & Boistel, 1971; Callec, 1974), 4-AP does not appear to have a cholinergic effect in the cockroach nerve cord (Hue et al. 1976) or the vertebrate end plate (Lemeignan & Lechat, 1967; Sobek et al. 1968). Nor does 4-AP appear to have anticholinesterase activity, as is indicated by *in vitro* experiments (Lemeignan & Lechat, 1967) and by the stability of the half-time for decay of postsynaptic potentials in the present experiments (Figs. 2–4). The present results also indicate that an effect upon postsynaptic membrane resistance, or equilibrium potential, is not involved. Thus, the most important synaptic action of $5 \times 10^{-5}$ M 4-AP seems to be at the presynaptic level.

The presynaptic effect appears to involve an increase in transmitter release with no increase in cercal fibre recruitment. 4-AP also appeared to induce action potentials presynaptically – the ‘antidromic’ spikes. After treatment of the spinal cord of the cat
with 4-AP, ‘antidromic’ potentials have been observed in the dorsal roots (Chanelet & Lemeignan, 1969; Lemeignan, 1973). Lemeignan (1973) has interpreted these potentials as resulting from presynaptic depolarization. In our experiments, such depolarization may have occurred, but, taking into account the observation that ‘antidromic potentials’ appear even at concentrations which do not depolarize the postsynaptic membrane (10^-6 to 5 x 10^-5 M, Hue et al. 1975b), and by analogy with results on the axonal membrane (Pelhate et al. 1974b), we think it more likely that there is an increase in excitability of the presynaptic membrane. This increase may at least partly account for the increased transmitter release.

Increased transmitter release would also be effected by 4-AP as follows. Lengthening the depolarization of the presynaptic membrane at the neuromuscular junction increases transmitter release (Katz & Miledi, 1967a). Since 4-AP increases action potential duration by selective blockage of K^+ channels in giant axons of the cockroach (Pelhate et al. 1974b) and the presynaptic fibre of the squid giant synapse (Llinás et al. 1976), it is conceivable that the presynaptic action potential is lengthened, and that this induces an increase in transmitter release. The same mechanism has been proposed for the action of TEA at the squid giant synapse (Katz & Miledi, 1967b; Kusano, Livengood & Werman, 1967) (TEA also blocks K^+ channels, e.g. in the cockroach; Pichon, 1969).

We wish to thank Mr L. Mony for his helpful advice. Mrs A. Fuentes, J. Le Guen and Mr M. Bedouet provided technical help. This work was carried out with the participation of the Association pour la Recherche Neurophysiologique et Neuropharmacologique en Pays de Loire.

REFERENCES


Action of 4-aminopyridine in the cockroach


