Modulation of transmission in different electronic junctions by aminopyridine

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Summary. 4 distinct electronic inputs can be identified in amphibian motoneurons perfused with calcium-free solution containing manganese. The differential effect of 4-aminopyridine on different electrotonic junctions may reflect the peculiarities of molecular architecture of potassium channels in different electrically excitable presynaptic membranes.

In contrast to synapses with chemical mode of transmission in electrotonic junctions, the generator of the postsynaptic current is a) located within the presynaptic membrane and b) produced by the opening of the voltage-sensitive membrane channels. To find out more about the relation between the electrical events in the presynaptic membrane and electrotonic excitatory postsynaptic potential (EPSP), it is desirable to interfere with the changes of ionic conductance produced by the presynaptic impulse. In the present investigation, an attempt was made to affect the transmission process across the different electrotonic junctions of amphibian motoneurones by 4-aminopyridine (4-AP) which blocks voltage sensitive K^+ currents in many neural tissues[2–5], including frog peripheral nerve[6].

The effect of 4-AP on the intracellularly recorded EPSPs in solutions containing no Ca^{2+} but 2 mM Mn^{2+} (standard saline), or 1.5 mM Ca^{2+} and 2 mM Mn^{2+}, was tested on the isolated perfused frog spinal cord (*Rana ridibunda*). Pretreatment with Mn^{2+} quickly and reversibly abolished chemically mediated synaptic transmission, whereas electrotonic EPSPs elicited by stimulation of dorsal and ventral roots (DR and VR) or medullary reticular formation (RF) were not altered after 10–14 h of perfusion. 4-AP (4-aminopyridine purum, Fluka AG, $3 \cdot 10^{-5}$– to $5 \cdot 10^{-4}$ M) quickly increased the duration, peak amplitude and time to peak of DR EPSP (figure 1). This effect was dose-dependent and in 0.5 mM 4-AP the total duration of DR EPSP could reach 300–350 msec (as compared with 30–40 msec before the treatment) and its amplitude – 4–5 mV. Simultaneously with the prolongation of the EPSP, numerous humps appeared (figure 1), suggesting the rhythmic firing in the presynaptic terminals. The antidromic spike was only moderately prolonged. Neither the resting membrane potential nor the postsynaptic membrane time constant was modified. There was no change in the average shape of the spontaneous miniature

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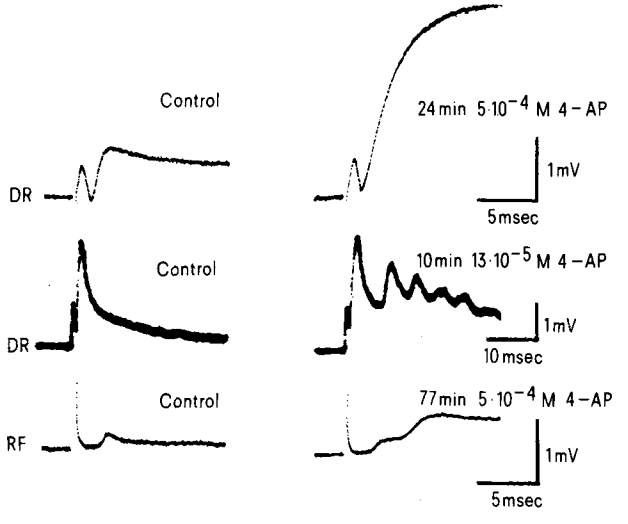

Fig. 1. Effect of 4-AP on electrotonic DR and RF EPSPs recorded from 3 different motoneurones. The top and the bottom traces are averaged records, obtained in Ringer containing 1.5 mM Ca^{2+}, 2 mM Mn^{2+}. The middle traces were recorded in standard saline (no Ca^{2+}, 2 mM Mn^{2+}). Temperature during recording of these and all other traces was 16–18 °C.

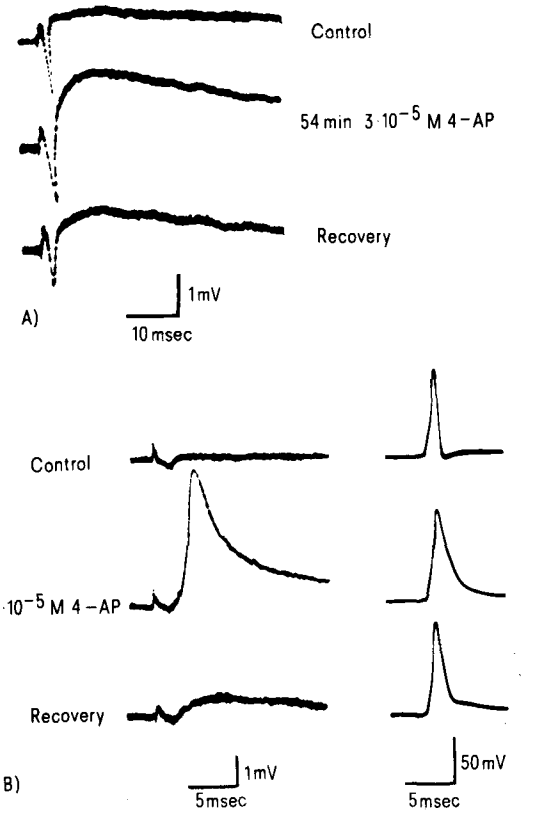

Fig. 2. *A* Records of early VR EPSP in standard saline (top), after addition of 4-AP (middle) and after washing for 70 min in standard saline (bottom trace). *B* Effect of 4 AP on late VR EPSP (left) and antidromic action potentials (right) recorded from the same motoneurone. Top are responses recorded after 135 min perfusion by standard saline, middle are responses obtained 20 min after addition of 4-AP, bottom are responses 54 min after washing in standard saline.

EPSPs. These results rule out the rather remote possibility that the alterations of DR EPSPs by 4-AP are due to its action on the postsynaptic membrane. The effects of small concentrations were reversible, while after long exposure to large concentrations of 4-AP we could not achieve the complete restoration. The rate of recovery of antidromic spike was clearly much faster. A similar dramatic increase of the RF EPSP by 4-AP was found (figure 1), although the latter were much smaller in amplitude and could be detected only in few motoneurones in contrast to electrotonic DR EPSPs which were present practically in all impaled cells.

The electrotonic VR EPSPs can be subdivided into 2 separate groups: early and late. The former are characterized by very short latency (0.5–2.0 msec) and relatively long duration. The subtraction of the extracellular response from the intracellular one demonstrates that the early VR EPSPs always begin before the peak of the antidromic spike. After 4-AP treatment, the early VR EPSPs were enhanced and prolonged in a similar way as DR and RF EPSPs (figure 2, A). This fact and the short latency of early EPSPs suggest that they are produced by monosynaptic connections between recurrent collaterals of motor axons and motoneurones.

In standard saline, the delayed VR EPSPs were present only in a few cells. They had a very fast time course, but always appeared after the peak of the antidromic spike (latency 2.5–4.8 msec). When 4-AP was applied, the delayed VR EPSPs appeared in most cells and their height could reach up to 8–12 mV. However, the duration was only slightly altered and they preserved the rapid spike-like shape (figure 2, B). The long latency of delayed VR EPSPs and their sudden appearance after 4-AP treatment are consistent with the prolongation of the soma-dendritic spike and suggest that this type of activity is transferred from motoneurone to motoneurone via electrically operating dendro-dendritic synapses.

Our results show that 4-AP is an efficient potentiator of excitatory transmission in different electrotonic junctions and that this effect is produced at the presynaptic level. The observed enhancement of electrotonic EPSPs by the substance blocking K^+ conductance further indicates that they are not caused by K^+ accumulation in perineuronal space. The differential effect of 4-AP on different electrotonic junctions may reflect the distinct structural organization of relevant synapses (axo-dendritic and dendro-dendritic), but may depend also on the peculiarities of molecular architecture of K^+ channels in different electrically excitable presynaptic membranes. Therefore, this substance may be used as a probe for analysis of presynaptic events which, as a rule, are not subject to direct electrophysiological investigation.

Projections from nucleus accumbens to globus pallidus and substantia nigra in the rat[1]

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Summary. Stimulation of nucleus accumbens produces inhibition and facilitation of neuronal activity in the ipsilateral globus pallidus and substantia nigra. Small lesions in the accumbens reduce pallidal but not nigral GABA content.

Recent studies using the orthograde transport of labelled amino acids or the retrograde transport of horseradish peroxidase have shown that neurones of the nucleus accumbens send efferent projections to the globus pallidus and substantia nigra[2–4]. This report provides electrophysiological confirmation for these projections and discusses the possible neurotransmitters released.

Materials and methods. Experiments were performed in 10 male albino rats lightly anaesthetized with urethane (1.2 g/kg, i.p.). The effect of nucleus accumbens stimulation was examined on spontaneous neuronal activity in the ipsilateral globus pallidus and substantia nigra. The centre barrel of a multibarrelled micropipette was used to record extracellular action potentials. Other barrels contained aqueous solutions of substances for electrophoresis; pontamine blue, GABA, dopamine, 1 M NaCl (current control). Methods for recording and analysis have been described[5].

In other animals (11) unilateral electrolytic lesions (300–400 μA for 20 sec) of the nucleus accumbens were made. The concentration of GABA[6] was estimated in globus pallidus and substantia nigra 14 days afterwards.

Results. The effects of nucleus accumbens stimulation are summarized in the table. In the globus pallidus 39 of 62 cells tested were activated. The predominant response was inhibition of firing (33 cells) (figure 1). This occurred alone with relatively short and constant latency (3.3 msec), but in other cells the latency was longer (13.0 msec) more variable and inhibition was of shorter duration (table). In 2 cells short latency inhibition was followed by a single phase of rebound excitation. In 3 cells facilitation preceded a period of inhibition (figure 1) and 6 cells responded by facilitation alone. Facilitation was characterized by a burst of action potentials (figure 1) and no evidence was obtained for antidromic activation. The latency of these excitations was similar (2.4 msec) but the duration of excitation occurring alone was longer (45.0 msec) than that which preceded a period of inhibition (15.7 msec). Histological examination revealed that most cells affected (22 inhibited, 5 excited) were localized rostromedially in the ventral two-thirds of the pallidus. Both GABA and DA (5–75 nA) depressed all cells tested in the pallidus (26 cells).

In the substantia nigra 13 of 30 cells tested responded. Only simple inhibition (7 cells, figure 2) or simple facilitation (6 cells) was observed. The latency of inhibition (6.7 msec) or excitation (5.2 msec) was similar as were the respective durations of the evoked effect (table). Of the responsive neurones, most (5 inhibited, 5 excited)

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