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4-Aminopyridine and fiber potentials in rat and human hippocampal slices

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Summary. Compound fiber action potentials of stratum radiatum afferents in slices from human and rat hippocampus are shown to be prolonged by 4-aminopyridine (4-AP). This action can explain the enormous increase in synaptic transmitter release caused by 4-AP.

4-Aminopyridine (4-AP) and some related compounds increase synaptic transmitter release in the peripheral and central nervous systems[1-5] presumably through a block of potassium channels leading to a prolongation of action potentials in unmyelinated nerve fibers and terminals[6,7]. Such an action may be beneficial in demyelinating diseases and some other pathological conditions, and some promising clinical tests have indeed been performed[4,6]. In hippocampal slices of the rat, we have recently shown that 4-AP increases excitarory and inhibitory transmitter release without changing the electrical characteristics of the postsynaptic membrane[1,8]. Similar results were obtained in ganglia[9] olfactory cortex[10] and the dentate area of the hippocampus[11]. At a concentration of 10^{-5} M, 4-AP caused spontaneous, sometimes seizure-like discharges and an increase of synaptic potentials (spontaneous and evoked) without altering the time course of intracellulary measured action potentials and extracellulary recorded population spikes of CA 1 pyramidal cells. The epileptiform activity is not due to a GABA antagonism[1,12]. The input volley, which is a summed action potential of afferent fibers activated by electrical stimulation, was also unchanged. If spike broadening occurred in the terminal regions of the afferent fibers it should be detected as a prolongation of the input volley. Since this potential is small, and often merges with the much larger synaptic potential under normal conditions we have now investigated the action of 4-AP in conditions where synaptic transmission was blocked. In this way, much larger and purer fiber potentials could be studied without contamination by synaptic effects.

Our methods for preparing and maintaining brain slices have been described previously[1,13]. 450-µm-thick slices were cut from the hippocampi of 11 rats and a human hippocampus, removed by hippocampectomy for treatment of drug-resistant limbic epilepsy[14]. The slices lay on a nylon mesh, in a triple version of our perfusion chamber, were

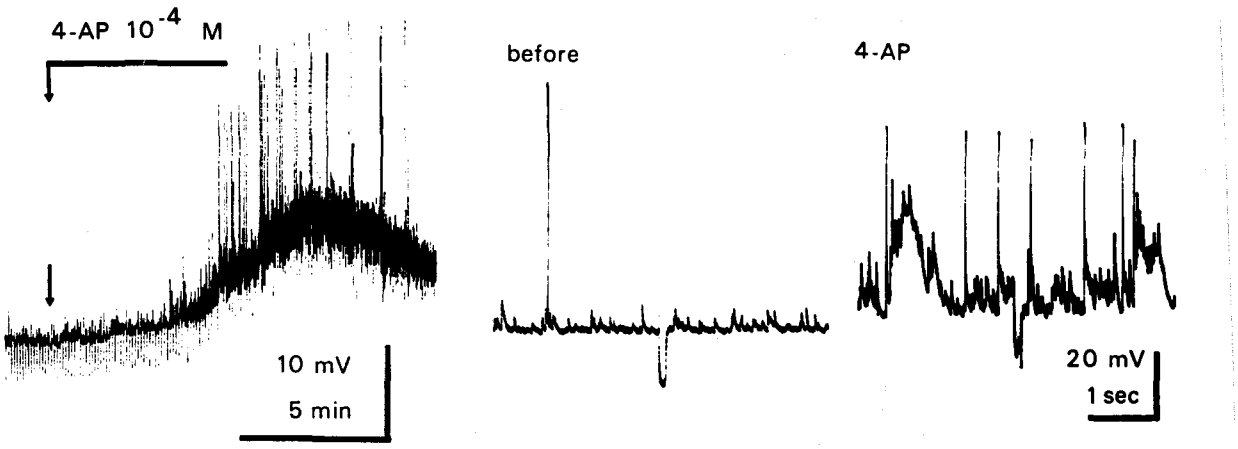

Figure 1. Action of 4-aminopyridine (4-AP) on a CA 1 pyramidal cell in the hippocampus, recorded intracellulary with a KCl-filled microelectrode (all synaptic potentials are depolarizing). 4-AP causes a depolarization of the membrane potential (left trace, which is from a pen recorder with low frequency response), an increase in synaptic and action potentials (thickening of left trace) and depolarizing shifts (slow upward deflections in left and right trace). Downward (negative) deflections are from hyperpolarizing current injection.

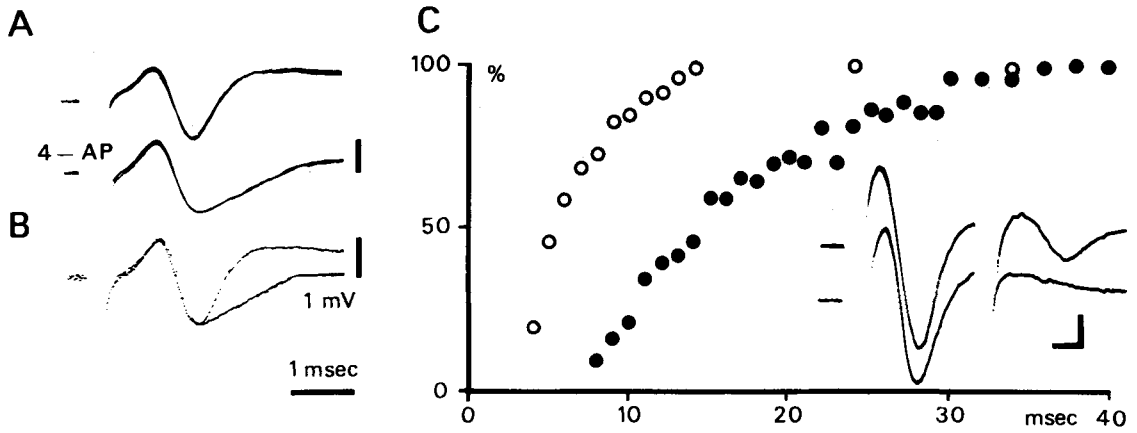

Figure 2. Effect of 4-AP on fiber potentials in slices from rat (A, and human (C) hippocampus. *A* Several superimposed traces of stratum radiatum fiber volleys before and during 4-AP (10^{-4} M) recorded in a solution containing 0.2 mM Ca and 4 mM Mg. *B* Averaged traces (8 sweeps) before and during 4-AP photographically superimposed. *C* Absolute and relative refractoriness tested by double shocks at varying distances. Open circles: control, filled circles: 4-AP 10^{-4} M. Ordinate: normalized fiber volley amplitude, abscissa: inter-stimulus interval. Insert shows fiber volleys evoked at a distance of 4 msec before (upper trace) and during (lower trace) 4-AP 10^{-4} M. Calibration 1 mV, 1 msec.

under- and perifused at 32 °C with oxygenated artificial cerebrospinal fluid and superfused with warmed, moistened 95% O_2/5% CO_2 gas mixture. Conventional methods were used for stimulation and recording. Synaptic transmission was blocked by adding magnesium (Mg, 4 mM) and reducing calcium (Ca, 0.2 mM) or incubation of the slices in non-oxygenated perfusion medium for 30 min. The 3 slices from a human hippocampus displayed good fiber potentials but no synaptic transmission, presumably because of oxygen deprivation during the operation. Similar results were obtained in all these conditions.

The typical action of 4-AP on the hippocampus with inact synaptic transmission is illustrated in figure 1. Intracellular recording was performed with a KCl-filled microelectrode in order to visualize all synaptic potentials as depolarizing events[1]. Fiber volleys of 1-12 mV amplitude were recorded in the stratum radiatum of the CA 1 area after bipolar electrical stimulation in the same layer (0.2 msec, 70-150 μA) 0.5-1 mm apart from the recording electrode. Size and half falling times (the time from peak to half size) of the population action potentials were determined from averaged records before and after a 20-min exposure to 10^{-4} M 4-AP. In all 11 slices studied in such a way, the duration of the fiber potential was clearly prolonged. Half falling times increased from 0.32 ± 0.05 (SD) msec to 0.75 ± 0.17 (SD) msec, while the size of the volley was slightly increased. An increase up to 30% occurred in 6 slices, a decrease of 10% in one and no change was noted in 4 slices). One of the latter is illustrated in figure 2A, B.

The peak latency was delayed in 5 slices by up to 0.5 msec. Prolongation of the action potentials was also accompanied by a marked increase in refractoriness. This was measured by applying double shocks with varying distance through the stimulating electrode. One such experiment in a slice from the human hippocampus is illustrated in fig. 2C. Absolute refractoriness of the volley was increased during 4-AP (10^{-4} M) from 2.8 ± 0.5 (SD) to 6.6 ± 1.4 (SD) msec (5 slices).

Many of the investigated fibers are unmyelinated and also the myelinated ones give off unmyelinated branches which make synaptic contacts with the dendritic trees of CA 1 pyramidal cells[15]. The present results suggest that a broadening of the action potentials occurs during the time when 4-AP is present in the terminal branches of hippocampal afferent fibers. If 4-AP acts intracellularly, endings and small diameter dendrites are likely to be first affected; this would explain the lack of effect observed on cell body action potentials[1]. Alternatively, there may be a nonuniform distribution of 4-AP sensitive K-channels.

The action of 4-AP in rat and human hippocampus described here is in keeping with the findings from a number of invertebrate and vertebrate preparations[1,6,9,10] showing that 4-AP blocks delayed rectifier currents[4]. Blockade of the potassium efflux prolongs the duration of the action potential and allows more calcium ions to enter the fiber through voltage sensitive calcium channels[4]. This explains the enormous increase in transmitter release and consequently the synaptic potentials.

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