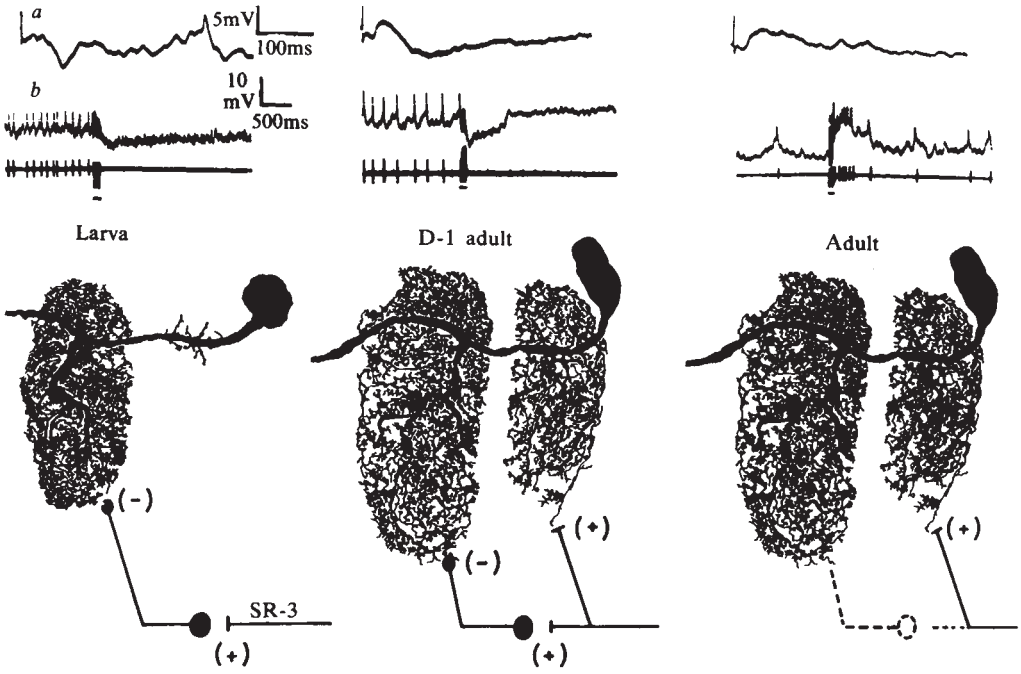

Fig. 3 Model for the changes observed in the interaction between MN-1 and SR-3. *a*, Response of MN-1 to single stimuli delivered to the contralateral SR-3. *b*, Response of MN-1 to a burst of stimuli (100 Hz) to the contralateral SR-3, which mimics the response of the receptor to natural stretch. Intracellular (top) and extracellular (bottom) records from MN-1. In the larva MN-1 is inhibited by the contralateral SR-3 through a polysynaptic pathway. The processes of SR-3 do not cross the midline of the ganglion, and lie in a different region of neuropil from the main neurite of MN-1. Single stimuli evoked long-latency i.p.s.p.s. On the day before adult emergence (D-1 adult) SR-3 evoked a biphasic response in the contralateral MN-1; the original inhibitory path was co-activated with a new direct excitatory path. Bursts of receptor spikes, however, still inhibited MN-1. The new dendritic processes of MN-1 overlapped with those of SR-3. After adult emergence, the inhibitory component was lost, and the SR-3 evoked a pure depolarizing response in the contralateral MN-1.

The inhibitory interaction between SR-3 and MN-1 is permanently inactivated after adult emergence. However, a reversible inactivation of parallel inhibition might be applicable to other situations in which hormones or other factors alter markedly the function of a particular neural pathway.

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A transient outward current in a mammalian central neurone blocked by 4-aminopyridine

B. Gustafsson*, M. Galvan†, P. Grafe† & H. Wigström*

* Department of Physiology, University of Göteborg, PO Box 33031, S-400 33 Göteborg, Sweden
† Department of Physiology, University of Munich, Pettenkoferstrasse 12, 8000 Munich 2, FRG

It is becoming increasingly clear that nerve cells in the mammalian central nervous system (CNS) have a very complex electroresponsiveness. They exhibit not only time- and voltage-dependent Na^+ and K^+ conductances, analogous to those in the squid giant axon[1], but also a variety of other conductances that have a significant role in the control of cell excitability. Of the outward currents, there are, in addition to the delayed rectifier, the Ca^{2+}-activated K^+ current[2,3] which underlies the long-lasting spike afterhyperpolarization, and the M current[4], a non-inactivating K^+ current evoked by membrane depolarization and blocked by muscarinic, cholinergic agonists. We demonstrate here the existence in a mammalian central neurone (hippocampal CA3 pyramidal cells) of yet another outward current, which is transient and may be carried by K^+ ions. Further, the experiments show that this current is substantially reduced by the convulsant 4-aminopyridine (4-AP)[5], resulting in a marked increase in cell excitability.

The experiments were performed on transverse slices (400–600 μm thick) of guinea pig hippocampus. The slices were kept either half or totally immersed (see below) in an experimental chamber perfused with a solution containing (in mM): NaCl, 123; KCl, 3.0; $CaCl_2$, 2.0; $MgSO_4$, 2.0; $NaHCO_3$, 26; glucose, 10. The temperature was kept constant at either 33 °C or 26 °C. Neurones in the CA3 region were impaled with 3 M KCl-filled microelectrodes and voltage-clamped using a single electrode switched clamp circuit (a DAGAN 8100 or a custom-built amplifier of similar design[6]); the switching frequency was 3 kHz and the duty cycle was 10–25%. Possible artefacts involved in the use of these instruments have been discussed elsewhere[7]. Drugs were applied in fixed concentrations to the bathing solution and the data collected were either computer-averaged or photographed from the oscilloscope screen using Polaroid film.

Figure 1*a* illustrates that in control solution, constant-current pulses applied to CA3 neurones at a resting potential of −70 mV evoked electrotonic potentials (ETPs) which were smaller in the depolarizing than in the hyperpolarizing direction. Closer examination of these depolarizing ETPs shows that they increased rapidly, then suddenly levelled off in a manner which suggests that an opposing outward current had been activated. This behaviour was more clearly seen when Na^+ and Ca^{2+} action potentials were blocked by addition of tetrodotoxin (TTX) and Mn^{2+} ions (Fig. 1*b*). A similar outward-going rectification (albeit at more depolarized membrane potentials) has been observed in sympathetic and hippocampal CA1 neurones and attributed to depolarization–activation of M channels[4,8,9]. However, addition of muscarine in sufficient concentrations to block M channels, did not noticeably affect the observed outward rectification (Fig. 1*c*). Thus it seems that, in these

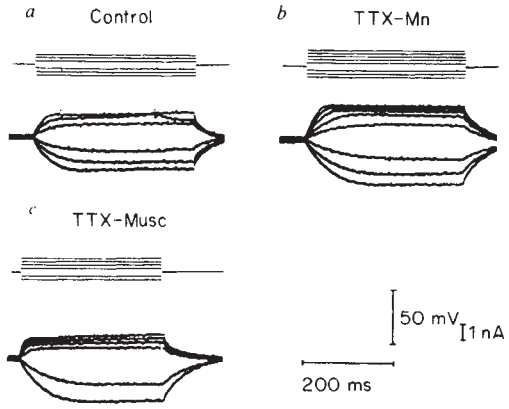

Fig. 1 Recordings of electrotonic potentials (ETPs) in CA3 neurones at 26 °C. *a*, Control solution; *b*, in solution containing 3 μM TTX and 4 mM $MnCl_2$; *c*, in solution containing 1 μM TTX and 25 μM muscarine chloride. (Slices were kept totally immersed in the solutions.) The membrane potential was set at −70 mV by passing steady d.c. current through the recording microelectrode, and the amplifier was set in the switched (sample-and-hold) current clamp mode. The top traces show rectangular constant current pulses and the lower traces the resulting membrane potential deflections. *a* and *b* were recorded from the same cell and illustrate the marked attenuation of the amplitude of depolarizing ETPs. Note the slow depolarizing 'creep' of the second largest depolarizing ETP leading to an action potential towards the end of the pulse. This creep was also often observed in the presence of inward current blockers. As judged from the amplitude of the smallest depolarizing and hyperpolarizing pulses, membrane resistance increased in the TTX–Mn solution, possibly due to blockade of tonic synaptic activity. *c* was recorded from another neurone and illustrates that the outward rectification was still present after blockade of possible M-currents by muscarine (Musc).

neurones, there is an outward rectifying current operating in the threshold range, that is distinct from M-channel activation.

Figure 2 shows voltage-clamp recordings from a CA3 neurone in a solution containing TTX. From a holding potential of −70 mV (Fig. 2*a*), hyperpolarizing voltages elicited small inward currents, which were relatively linear and time independent. When the cell was depolarized to potentials greater than −60 mV, a rapidly activating outward current was observed, which decayed over the next several hundred milliseconds. The peak amplitude of this transient outward current was increased as the cell was stepped to more positive potentials. This current–voltage relationship is plotted in Fig. 2*c* (closed circles); it is nonlinear, indicative of voltage-dependent activation. It was also observed that the current amplitude varied with the holding potential, increasing with membrane hyperpolarization (−70 to −100 mV) and decreasing to zero at holding potentials more positive than −55 mV. Thus, when the same cell was held at −40 mV and subjected to step hyperpolarizations, only steps to potentials more negative than −55 mV were followed, on return to the holding potential, by a transient outward current (Fig. 2*b*). The relationship between the hyperpolarizing step and the resulting outward current is shown in Fig. 2*c* (open circles) and is an approximate measure of the inactivation characteristic of this current.

The transient outward current was observed in all CA3 neurones examined ($n = 35$), and had an amplitude of 4.2 ± 1.5 (±s.d.) nA ($n = 9$) as measured 30 ms following a 50–55 mV, 1–1.5 s hyperpolarizing voltage step from −30 mV, and back again. The activation kinetics were too rapid to be resolved using the single electrode clamp technique; our data show only that the current appears to have peaked within 10–15 ms. The inactivation consisted of an initial rapid phase followed by a very slow second phase, which lasted for up to several seconds (Fig. 2*b*); this form of decay could not be described by first-order kinetics.

A transient current having these characteristics has been described in invertebrate[10,11] and vertebrate[12] neurones and has been shown to be mainly carried by K^+ ions. In the present experiments, an increase in the external K^+ ion concentration (40–60 mM with totally immersed slices) always decreased or abolished the transient outward current. A clear reversal was not obtained but this may simply reflect poor diffusion of K^+ ions into the slice, resulting in only a small positive shift of the K^+ reversal potential. Outward rectification, probably caused by activation of the transient outward current, persisted in solutions containing Ca^{2+}-channel blockers (Mn^{2+} ions) (Fig. 1*b*). Under voltage-clamp, it was also observed that the current was not blocked by Mn^{2+}, although the activation–voltage curve seemed to be shifted ~10 mV in the positive direction. This is, to a lesser extent, evident also from Fig. 1*b*. Thus, the results demonstrate the presence of a transient outward current in CA3 neurones, which induces a marked rectification in the current–voltage relation at membrane potentials close to threshold. This current is probably a K^+-current that is not dependent on Ca^{2+} ion movements.

It has been shown previously that the convulsant agent 4-AP can (at mM concentrations) selectively reduce the amplitude of transient outward currents in invertebrate neurones[13] as well

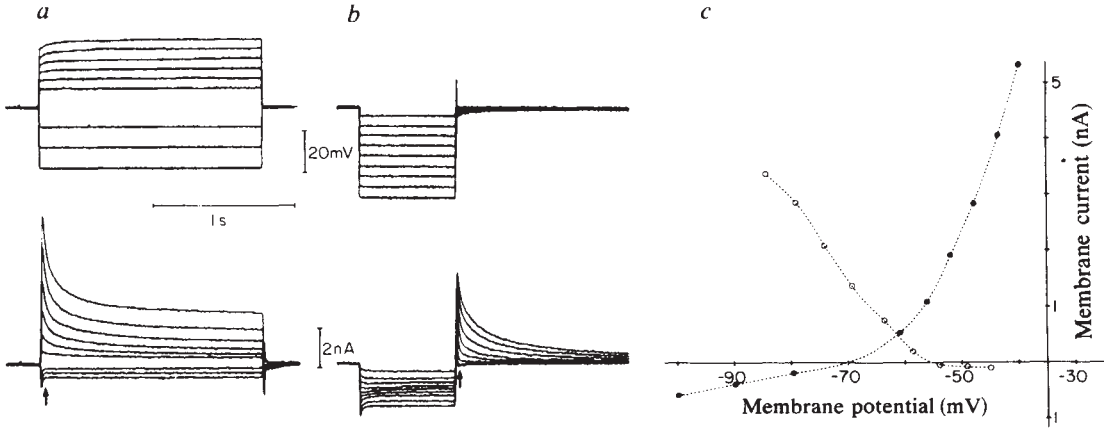

Fig. 2 Computer-averaged recordings of membrane voltage (upper traces) and current (lower traces) from a single CA3 neurone held at −70 mV (*a*) and −40 mV (*b*). Temperature, 33 °C. Slices were kept half-immersed in the solutions. Transient outward currents were elicited by step depolarizations from −70 mV or following hyperpolarizations from −40 mV. Note that for large pulses, the membrane voltage attained initially deviated from the command level. This results from inadequate feedback gain—a consequence of single electrode clamping with relatively high resistance microelectrodes. In *b*, small inward current relaxations can be observed during small hyperpolarizing commands, suggesting the presence of M-current. Measurements of membrane voltage and current, made at the times indicated by the arrows in *a* and *b*, respectively, are plotted in *c*. ●, The current–voltage curve from −70 mV; ○, the relationship between hyperpolarizing command potential and the resulting post-pulse current.

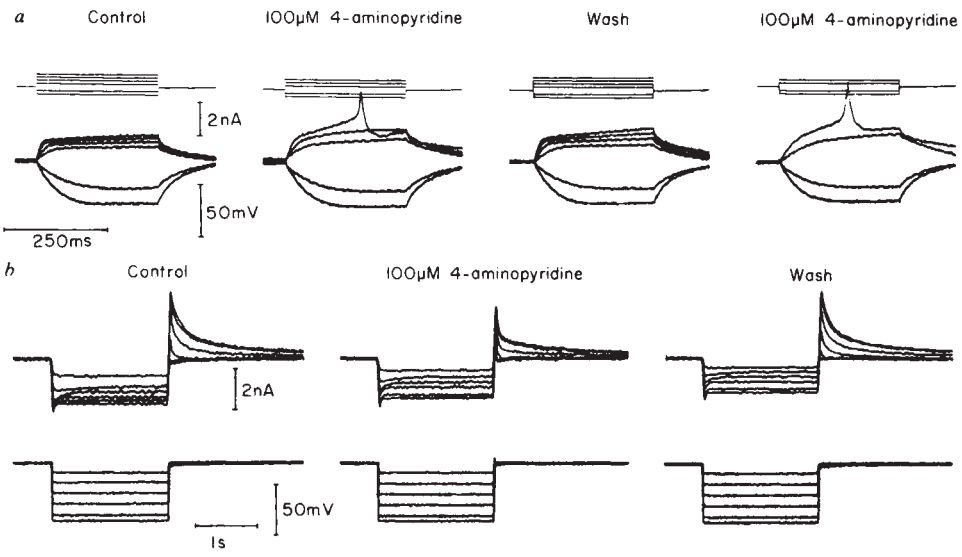

Fig. 3 Effect of 4-AP on electrotonic potentials (*a*) and transient outward currents (*b*) measured in two separate CA3 neurones. The experiment illustrated in *a* (26 °C) was performed in the presence of 1 μM TTX and 25 μM muscarine chloride; *b* (33 °C) was performed in 0.5 μM TTX. The slices in *a* were kept totally immersed, while those in *b* were kept half-immersed. In *a* the membrane potential was manually set at −70 mV; in *b* the holding potential was −30 mV. The current clamp records in *a* show electrotonic potentials before, at the end of and 60 min after bath application (8 min) of 100 μM 4-AP. After a substantial recovery had been obtained, 4-AP was applied again for a longer period (20 min). Note that a maximum effect was not achieved during the first application, as evidenced by the much larger change from outward rectification resulting from the second, longer application. The amplitude of hyperpolarizing ETPs was almost unaltered by 4-AP as was the current necessary to hold the membrane at −70 MeV. In *b*, the voltage clamp records illustrate that a brief (5 min) application of 100 μM 4-AP substantially and reversibly reduced transient outward currents.

as in mammalian cardiac muscle fibres[14]. Figure 3*a* demonstrates that in CA3 pyramidal cells, 100 μM 4-AP can reversibly reduce the outward rectification, resulting in a considerable increase in cell excitability; evidence for this comes from the appearance of a Ca^{2+} action potential. Furthermore, Fig. 3*b* shows that in voltage-clamp, the transient outward current was substantially blocked by 4-AP. These effects were evident after only a few minutes of 4-AP application at times when it was unlikely that the 4-AP concentration equalled that in the bathing solution. The classical K^+-channel blocker tetraethylammonium chloride (TEA), which blocks delayed rectifier channels[15] and Ca^{2+}-activated K^+ channels in vertebrate neurones[16], did not (at 3 mM) have any significant effect on this current. Note, however, that even in the presence of high concentrations (500 μM) of 4-AP (in which no transient outward currents were observed), some outward rectification from −70 mV remained. This may be related to the presence of M currents, which were also observed in these cells.

Thus, hippocampal CA3 pyramidal cells exhibit a transient outward current distinct from the delayed rectifier K^+ current, the Ca^{2+}-activated K^+ current and the M current. The activation–inactivation characteristics as well as the pharmacology are similar, in principle, to those of the so-called A current in invertebrate neurones and it seems reasonable to extend this terminology to mammalian central neurones. We cannot, however, be certain that the current described here is similar in all respects to that of invertebrates. Connor and Stevens[17] proposed that the A current controls repetitive firing in gastropod neurones. Its long duration and large amplitude in CA3 neurones also suggest that A currents in these cells, together with the Ca^{2+}-activated K^+ conductance, could serve such a role. More importantly, our data show that A currents are capable of controlling the approach of membrane potential to spike threshold and thus contribute to the control of cell excitability. The activation kinetics suggest that A currents may even affect the action potential (see ref. 18) and excitatory postsynaptic potential trajectories. The postsynaptic excitatory effect of 4-AP can probably be explained by blockade of A currents, although other contributory mechanisms cannot yet be excluded[19]. The presence of such currents also in vertebrate nerve terminals might explain the facilitatory actions of aminopyridines on neurotransmitter release[19]. Nevertheless, it certainly seems that the postsynaptic effects of 4-AP may contribute to its convulsant action.

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Serotonergic denervation partially protects rat striatum from kainic acid toxicity

Michael Berger, Günther Sperk & Oleh Hornykiewicz

Institute of Biochemical Pharmacology, University of Vienna, Borschkegasse 8a, A-1090 Vienna, Austria

We report here observations indicating that the rat striatum can be partially protected from the neurotoxic action of locally applied kainic acid (KA, a rigid structural analogue of glutamic acid[1]) by procedures resulting in reduced serotonergic brain activity. This observation may be important because the striatal damage produced by KA shows morphological and neurochemical similarities to the changes seen in the striata of patients dying of Huntington's chorea[2,3], an hereditary brain disorder, and because functional and morphological brain changes induced by KA have been proposed as a close model for human epilepsy[4]. Our observations thus suggest that serotonergic brain mechanisms influence the development of neuropathological changes accompanying certain neurological disorders.