Neuropsychobiology

Neuropsychobiology 2000;42(suppl 1):28–32

Modulation of Neural Cell Membrane Conductance by the Herbal Anxiolytic and Antiepileptic Drug Aswal

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Key Words

Aswal · Anticonvulsant · Calciumantagonism · Anxiety · Bipolar disorder

Abstract

To evaluate the effects of aswal on ionic fluxes and neuronal excitation, we performed extracellular and whole cell patch clamp recordings on CA1 pyramidal neurons of guinea pigs and Long-Evans rats. Aswal (100–250 mg/l) was administered systemically, and its effects on the rate of synchronized extracellular field potentials (EFP), membrane parameters, action potentials and postsynaptic potentials were recorded. The extracellular results obtained are consistent with calcium antagonistic properties. Intracellular recordings suggest that a direct sodium antagonistic effect as seen in many antiepileptic drugs plays no significant role. Further effects on ligand gated ion channels are discussed controversially. In summary, the cellular action of aswal appears heterogeneous with calcium antagonism playing a prominent role in counteracting excitation which may be a common feature in epilepsy and different psychiatric conditions as mood and anxiety disorder.

Introduction

Herbal remedies such as St John's wort play an increasing role in modern Western medicine due to their popular demand by patients. Traditional experience and small clinical trials suggest efficacy mostly in minor states of anxiety, depressed mood and sleep disturbances. Besides this, two drugs have also demonstrated anticonvulsant activity, thus making them obviously potent CNS depressant substances: The kava-pyrone kawain and aswal, prepared from dried roots of *Withania somnifera*, a traditional Indian remedy which has recently been released in Switzerland. For kawain, we previously described its effects on ionic membrane fluxes [1, 2].

W. somnifera (winter cherry) is an indigenous plant of India. Constituents of the plant, roots and leaves, are widely used in Ayurveda and Siddha medicine as a sedative and aphrodisiac. It has also been traditionally used in treating epilepsy. Furthermore, a variety of cerebral and immunomodulatory (antitumor) effects have been described [3, 4]. Antioxidant effect of active components of *W. somnifera* may explain, at least in part, the reported antistress, immunomodulatory, cognition-facilitating, anti-inflammatory and antiaging effects produced by

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them in experimental animals [5]. In behavioral tests, antistressor capabilities of aswal have been described [6]; with chemical stressors, simultaneous oral administration of ashwagandha (100 mg/kg) prevents the rise in lipid peroxidation in stressed rabbits and mice after intravenous administration of 0.2 μg/kg of lipopolysaccharide from *Klebsiella pneumoniae* and 100 μg/kg of peptidoglycan from *Staphylococcus aureus* [7].

At least on the behavioral side, a common mechanism producing these effects can be a modulation of membrane ion channel function, similar to kava-pyrones. To further evaluate the effects of aswal on ionic fluxes and neuronal excitation, we performed the following experiments.

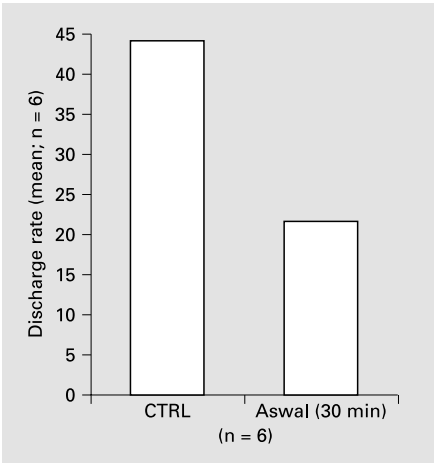

Fig. 1. Graphic presentation of the reduction in the frequency of EPS in the hippocampus by aswal (100 mg/l).

Methods

Extracellular Recordings

The experiments were carried out in hippocampal slices of guinea pigs (300–400 m thick). The brain was removed from the guinea pig under ether anesthesia. The slices were preincubated for 2 h in a 28 °C standard saline containing (in mmol/l) NaCl 124, KCl 4, $CaCl_2$ 0.75, KH_2PO_4 1.24, $MgCl_2$ 1.3, $NaHCO_3$ 26 and glucose 10, and which was equilibrated with 5% CO_2 in O_2 (carbogen). After preincubation, the slices were transferred to a recording chamber which was continuously (2 ml/min) superfused by 32 °C saline. The ionic composition was the same as during preincubation except for the Ca^{2+} concentration which was raised from 0.75 to 1.75 mmol/l and the K^+ concentration which was augmented from 4 to 8 mmol/l [8, 9]. Slices were perfused for a minimum of 1 h with this standard solution before switching to the zero Mg^{2+} solution. The pH of the carbogen-equilibrated saline was 7.4 and did not change when $MgCl_2$ was omitted or when drugs were added to the saline. Aswal (100–250 mg/l) was dissolved in DMSO in a concentration of 0.1% which had no effects on the tested field potentials by itself.

Extracellular recording were performed from stratum pyramidale of CA1 and CA3 areas of the hippocampal slice using glass microelectrodes (resistance was in the range of 1–3 MΩ) filled with 2 mol/l NaCl. Signals were amplified by a conventional microelectrode amplifier (NPI electronics, Tamm, Germany), stored on an oscilloscope and plotted on a pen recorder.

Intracellular Recordings

Whole cell patch clamp recordings from the CA1 region of rat hippocampal slices were performed. A bipolar stimulus electrode was placed in the stratum radiatum. Slices were prepared from rats of both genders, aged 25–40 days, and maintained using standard procedures. Rats were decapitated under halothane anesthesia, the brains were rapidly removed, and 300- to 400-μm-thick transverse slices were cut from the hippocampus with a vibratome (Model 820; Spencer Inc.). Slices were then placed in oxygenated artificial cerebrospinal fluid at room temperature. Whole cell recordings were obtained with the technique of Blanton et al. [10]. Briefly, borosilicate glass electrodes (resistance 4–6 MΩ) were filled with 100 m*M* potassium citrate, 20 m*M* KCl, 1 m*M* $CaCl_2$, 3 m*M* $MgCl_2$, 2 m*M* MgATP, 2 m*M* NaGTP, 3 m*M* EGTA and 40 m*M* Hepes. Recordings were made with an Axopatch 200A amplifier (Axon Instruments, Burlingame, Calif., USA) using the p-clamp 6.0 software. Artificial cerebrospinal fluid contained (in m*M*): NaCl 124; KCl 3.75; KH_2PO_4 1.25; $MgCl_2$ 1.3; $CaCl_2$ 3.5; $NaHCO_3$ 26, glucose 10; bubbled with 95% O_2-5% CO_2 and maintained at 30 ± 2 °C throughout the recordings.

Results

Extracellular Recordings

During perfusion of the slice with a zero Mg^{2+} solution, typical field potentials developed within a few minutes, being the correlate of intracellularly recorded paroxysmal depolarization shifts [11]. Several investigations have shown that calcium currents essentially contribute to the generation of these EFP [8, 9, 12]. There is no difference in the frequency of occurrence of EFP between CA3 and CA1 regions of the hippocampal slice [12]

The systemic administration of aswal (100 mg/l) with the zero Mg^{2+} solution resulted in a reduction in the repetition rate of field potentials of 56% after 30 min (fig. 1)

Intracellular Recordings

Stable whole cell patch clamp recordings were established using standard methods. Only cells with a resting membrane potential between –58 and –65 mV and an input resistance of 50–100 MΩ were used. Stability of these parameters was checked several times during a 15-min baseline recording. Cells were classified as pyramidal-type cells by their electrophysiological properties, in-

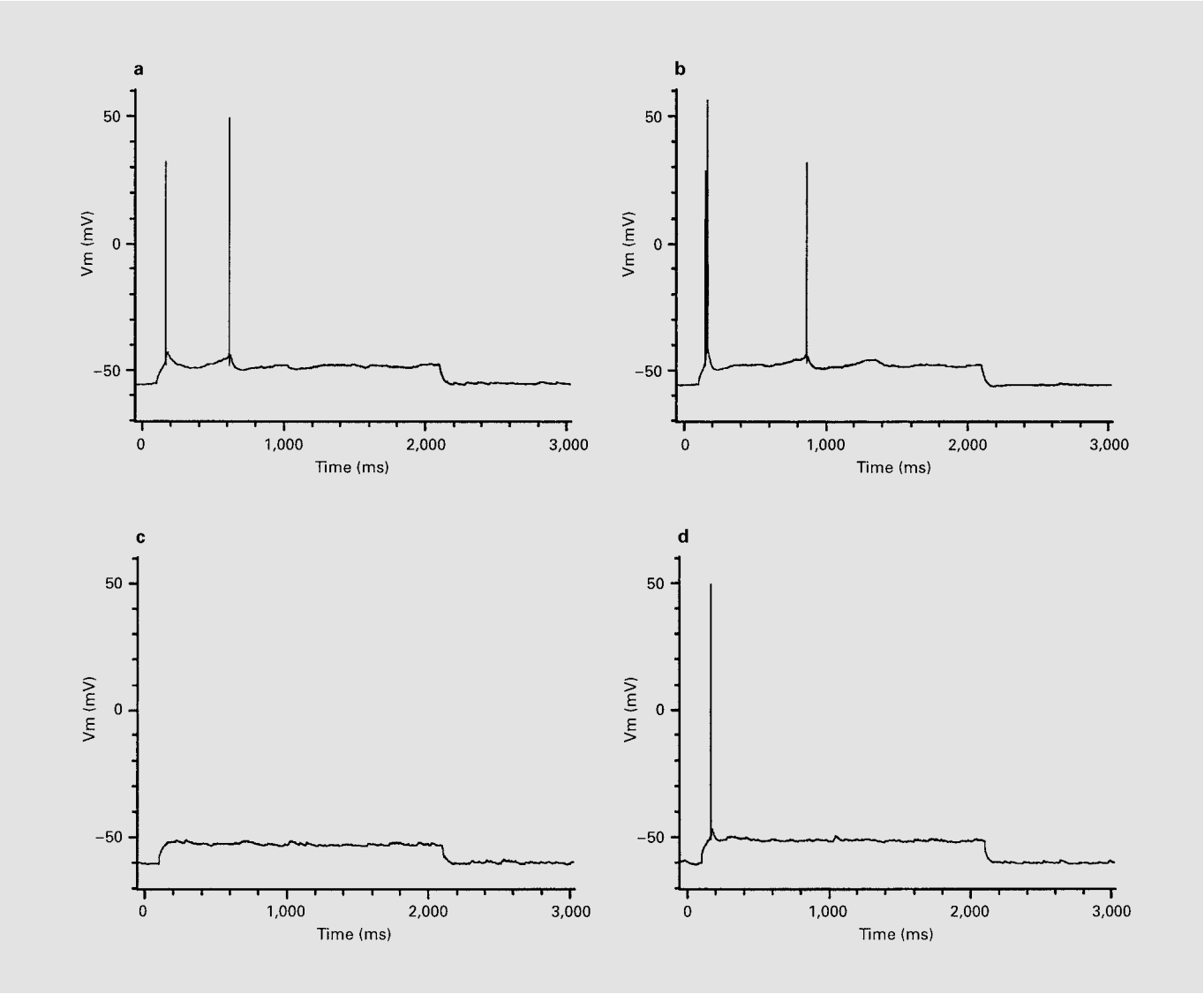

Fig. 2. Reduction in AP frequency in response to a depolarizing current injection. **a** Baseline. **b** During superfusion with aswal (250 mg/l) for 15 min. **c** After 30 min aswal. **d** After 15 min washout.

cluding action potential duration and spike accommodation with repetitive firing. Aswal (100 or 250 mg/l) was added to the superfusate for 30 min, followed by a 15-min washout period.

We found that:

(1) The resting membrane potential, action potential (AP) threshold and input resistance remained unchanged with aswal in both concentrations tested (100 and 250 mg/l).

(2) When repetitive AP firing was elicited by injecting currents from 150 to 450 pA, the effect of aswal on repetitive firing in response to current injection was heterogeneous; 2/5 cells showed a small reduction (fig. 2), 2/5 an increase of the frequency, and 1/5 was not affected. The AP amplitude remained unchanged in all cells.

(3) Finally, excitatory (EPSP) and inhibitory (IPSP) postsynaptic potentials were evoked by stimulation of the stratum radiatum. Recording postsynaptic potential in 5 cells, we saw in 4/5 cells a 38 ± 20% reduction in the EPSP with aswal, but no effect on IPSP expression (fig. 3).

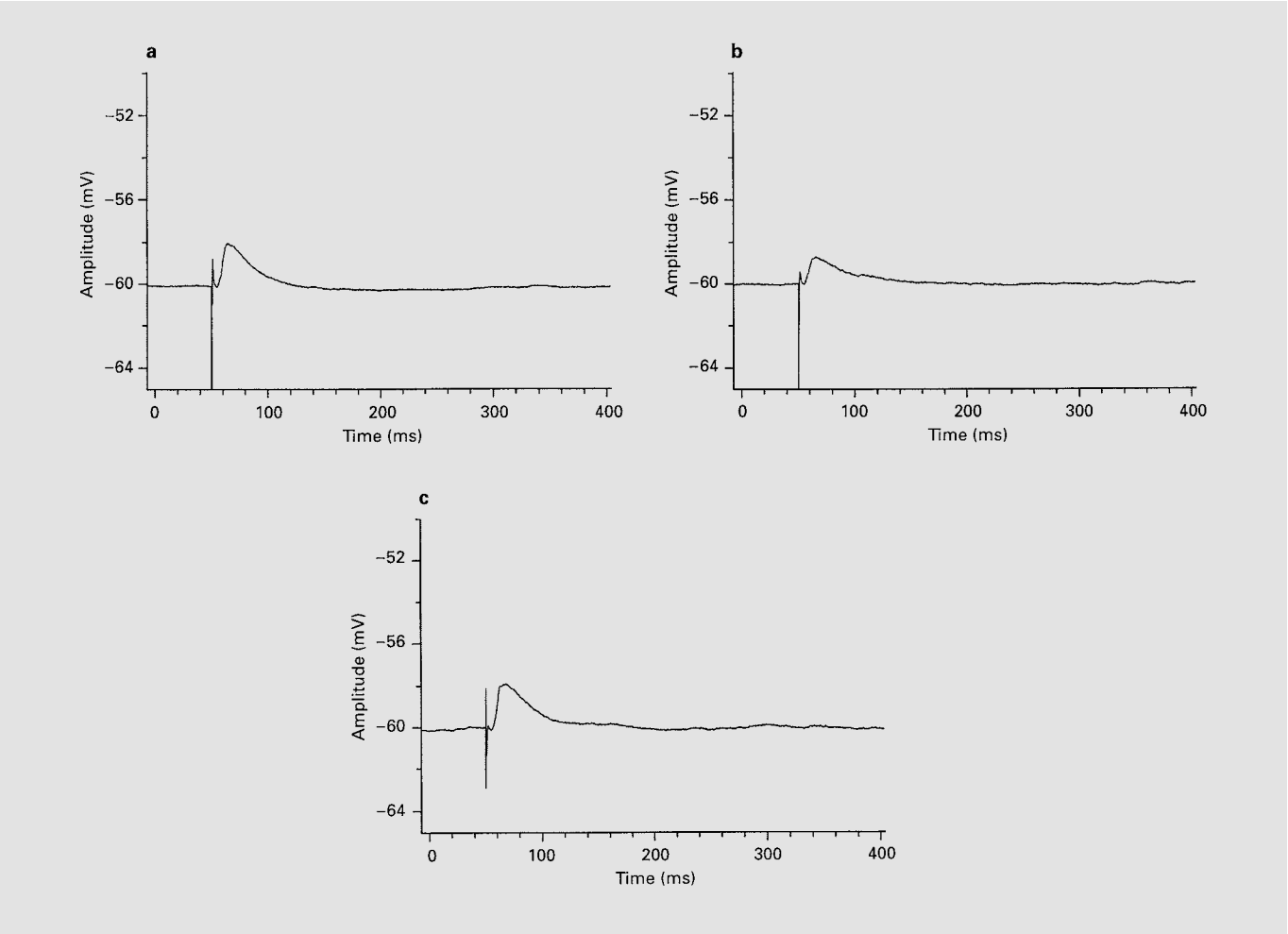

Fig. 3. Reduction in the EPSP amplitude. **a** Baseline. **b** Aswal 100 mg/l. **c** After 15 min washout.

Discussion

The zero-Mg^{2+}-induced field potential changes are thought to be generated due to the unblocking of NMDA receptors and the following activation of voltage-dependent calcium channels [8, 12–14]. Moreover, they are blocked by organic calcium channel blockers [9, 12]. Our extracellular results are also consistent with calcium antagonistic properties of aswal. As a fact, calcium antagonists are effective antiepileptic drugs in vitro and can exert mood-stabilizing properties in patients [15]. Thus, calcium antagonism may be an mechanism underlying the action of aswal. Similar to anticonvulsants with calcium antagonistic features, and to nimodipine, aswal exerts also antikindling properties in vivo [16]. Our intracellular recordings showing no reproducible reduction in repetitive AP firing and AP amplitude suggest that a direct sodium antagonistic effect as seen in many antiepileptic drugs plays no significant role.

GABAergic properties mediated through the barbiturate binding site have been implicated for the antiepileptic potency of aswal [17]. In our experiments, however, the stability of the resting membrane potential while aswal was administered makes at least a strong GABAergic component of its action unlikely, as direct application of GABA or its agonists drag the resting membrane potential towards more negative potentials. The lack of acute, strong GABA agonistic properties is also supported by the lack of changes in the IPSP following stratum radiatum stimulation. These results are supported by histochemical results of Schliebs et al. [18], showing effects on acetylcholinesterase, but not on glutamatergic or GABAergic mark-

ers. The observed EPSP reduction can also be related to the calciumantagonistic properties and not necessarily to antiglutamatergic mechanisms.

Taken together, the cellular actions of aswal appear heterogeneous with calcium antagonism playing a prominent role in counteracting excitation. A role of increased intracellular calcium concentration has been implied for such different conditions as epilepsy, alcohol withdrawal, bipolar disorder and anxiety. Thus, aswal may be a potentially effective drug for these disorder. At least on the cellular level, aswal seems to have a profile of action which can justify a further follow-up of its efficacy for mood disorder in controlled trials.

Acknowledgements

This study was supported in part by the Deutsche Forschungsgemeinschaft (DFG; GR 1264/4-1) and in part by the Vada and Theodore Stanley Foundation.

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