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PACEMAKER POTENTIALS ARE THE PHYSIOLOGIC BASIS OF EPILEPTIFORM ACTIVITY IN THE BUCCAL GANGLIA OF *HELIX POMATIA**

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Mechanisms of epileptic activity in nervous systems were studied using the identified neurons B1 through B4 in the buccal ganglia of the snail *Helix pomatia* as a model system. Activities were recorded with intracellular microelectrodes. Epileptiform activity was induced by bath application of an epileptogenic drug (pentylenetetrazol: 1 mM to 40 mM, or etomidate: 0.1 mM to 1.0 mM). Epileptiform potentials recorded from the somata of neurons consisted of paroxysmal depolarization shifts (PDSs). With increasing concentration of an epileptogenic drug, pacemaker potentials in neuron B3 developed into PDS. Simultaneously several types of chemical post-synaptic potentials were suppressed in amplitude. Since on the one hand epileptic seizures only appear when PDS are synchronized in many neurons and since on the other hand synaptic potentials were found to be suppressed during epileptic conditions, mechanisms underlying neuronal synchronization were studied. Evidence was found that, under epileptogenic conditions only, neurons were synchronized by an non-synaptic release of substances. Strong depolarizations accompanied by an increase in intracellular calcium concentration are known to induce an unspecific exocytosis. Thus, an unspecific exocytosis from the dendrites of PDS-generating neurons probably appears under epileptic conditions and synchronizes neighbouring neurons.

Keywords: Buccal ganglia – *Helix pomatia* – epilepsy – pentylenetetrazol – pacemaker potential – non-synaptic release

INTRODUCTION

Epilepsy is a common disease, about 5% of population has at least one epileptic seizure in life and a chronic epilepsy develops in about 1% of population. Epilepsy shows itself in epileptic seizures which may consist of epileptic convulsions. On the neuronal level, seizures are represented as paroxysmal depolarization shifts (PDS) [1, 9]. A PDS consists of a steep depolarization superimposed by action potentials, a plateau of 30 to 40 mV of amplitude and a steep repolarization. PDS result in convulsions when they are synchronized within a population of motor neurons. PDS

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have been recorded in many nervous systems and they appeared in the same shape in different types of seizures. Thus, PDS are the neuronal basis of epileptic seizures and epilepsy. Mechanisms of PDS are not fully understood [8].

It has been postulated many times that PDS represent "giant EPSP". According to this hypothesis, epileptic activity is thought to result from an imbalance of chemical synaptic excitation and chemical synaptic inhibition, i.e. from an increase in excitation and/or a decrease of inhibition. Although there has been some doubt concerning the "giant EPSP hypothesis", there is at present no alternative which could explain neuronal synchronization. The present observations allow an alternative explanation of PDS and of neuronal synchronization, i.e. PDS are "giant pacemaker potentials" and neuronal synchronization results from non-synaptic release of substances from the dendrites of an epileptically active neuron to the surface of neighbouring neurons.

MATERIAL AND METHODS

The buccal ganglia of *Helix pomatia* were isolated from the animal and kept in an experimental chamber continuously perfused with a solution which contained (in mM): 130 NaCl, 4.5 KCl, 9 $CaCl_2$, and 5 Tris-Cl. Solution was thermostabilized (19.5 °C to 20.5 °C) and pH adjusted (7.35 to 7.45). Several of the identified neurons B1 through B4 were recorded simultaneously with intracellular microelectrodes. For electrical stimulation a peripheral part of the anterior pharyngeal nerve was sucked into a fire polished glass electrode. Bipolar electric pulses lasting 2 ms were used [4]. Current injections were done via a second intracellular microelectrode. Epileptiform activity was induced by adding the epileptogenic drug pentylenetetrazol (1 mM to 40 mM) or etomidate (0.1 mM to 1.0 mM) [3] to the bath fluid.

RESULTS

During application of an epileptogenic drug, typical PDS appeared in neuron B3 (Fig. 1). The depolarization shifts are recorded from the soma of neuron B3. Along the axon, the depolarizations are transformed into high spike rates which can be recorded from the cerebrobuccal connective known to contain the axon of neuron B3. PDS appear spontaneously (i.e. without further stimulation) as long as the epileptogenic drug is applied (Fig. 2).

It was first tested whether chemical synaptic potentials are involved in the generation of PDS. The central pattern generator (CPG) for food uptake mechanisms is one source of synaptic potentials in the buccal ganglia. Neuron B4 has been shown to be a pharyngeal motor neuron [14] which receives strong synaptic activation from the CPG mentioned. Since neuron B3 is known to reliably generate PDS, neurons B3 and B4 were recorded simultaneously (Fig. 3). Figure 3 upper left recording shows the typical fluctuations of membrane potential of a neuron B4, which resulted from the synaptic activation from the CPG for food uptake. With increasing concentration

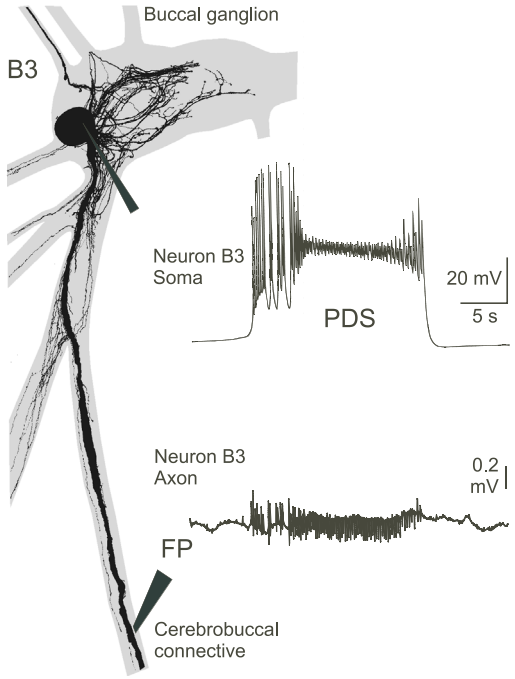

Fig. 1. Epileptiform activity recorded in neuron B3 in the buccal ganglia of *Helix pomatia.* Simultaneous recordings: intracellularly from the soma and extracellularly from the axon. PDS: paroxysmal depolarization shift, FP: field potential. Neuron B3 was intracellularly stained with cobalt-lysine, structures were reconstructed by drawing the fibers within the contour of the buccal ganglion and its nerves. Oscilloscope recordings

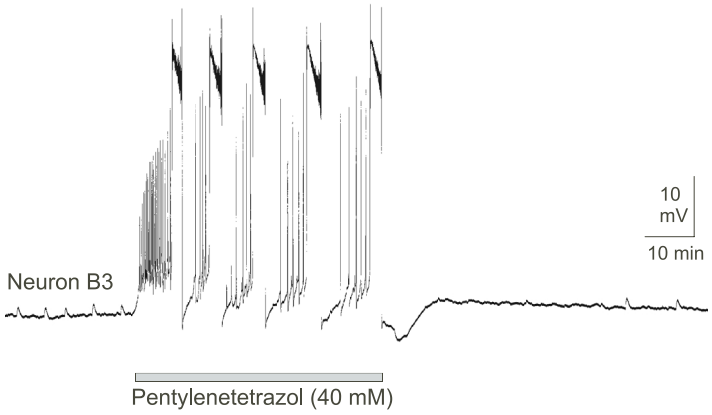

Fig. 2. Induction of "epileptogenic conditions" by bath application of an epileptogenic drug. The bar shows the duration of application of pentylenetetrazol. Pen recording, action potentials are distorted by the recording system

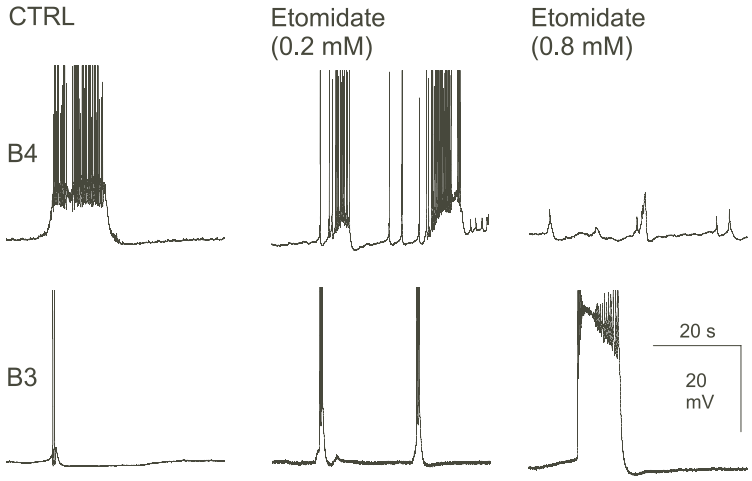

Fig. 3. Comparison of membrane fluctuations of neurons B4 and B3 in the buccal ganglia of *Helix pomatia* under control (CTRL) and "epileptogenic conditions" (etomidate: 0.2 mM and 0.8 mM). Oscilloscope recordings, action potentials are cut

of an epileptogenic drug, the synaptically evoked fluctuations in neuron B4 decreased (upper middle and upper right recordings). Simultaneous to the decrease of synaptic potentials in neuron B4, neuron B3 developed typical PDS (lower recordings of Fig. 3).

To further study the effects of epileptogenic drugs on synaptic potentials, a buccal nerve was stimulated electrically [2]. The synaptic responses evoked by this were also depressed with increasing concentration of the epileptogenic drug. With pentylenetetrazol (40 mM), IPSP were blocked completely and EPSP were decreased to 9% of control (average value, ranging from 37% to 0%, 14 experiments). Thus, whereas synaptic potentials of differential origin decreased during epileptogenic conditions, i.e. during application of an epileptogenic drug, pacemaker potentials in neuron B3 were found to increase (cf. Fig. 3, lower line).

To study the relation between pacemaker potentials and epileptiform activity, slow triangular currents were injected into the soma of neuron B3 under control and during application of an epileptogenic drug (Fig. 4). Under control conditions (CTRL), typical pacemaker potentials appeared in neuron B3 at membrane potentials of about –40 mV which was below normal resting membrane potential (marked by grey bars in Fig. 4, left collumn). As shown in Fig. 4 (right collumn, CTRL), neuron B3 was generally silent at resting membrane potentials. With increasing concentration of an epileptogenic drug several alterations were found: (1) activation range of spontaneously appearing potentials increased and reached normal resting potentials, (2) pacemaker depolarizations increased (Fig. 4, right column, marked dark grey), and (3) pacemaker repolarizations (marked light grey) were progressively delayed (marked by arrows). Thus, the depolarization of a PDS developed from the pace-

maker depolarization and PDS-repolarization corresponded to pacemaker-repolarization. In the used model nervous system, synaptic potentials were suppressed under epileptic conditions whereas pacemaker potentials, which are generated within the respective neuron, developed into PDS.

Epileptic seizures in man can only appear (e.g. as convulsions) when many neurons are synchronized. Since presently synaptic potentials were found to be depressed, the mechanisms of neuronal synchronization must be evaluated. In simultaneous recordings of several buccal neurons it was regularly observed that neurons which did not generate a PDS under epileptic conditions showed depolarizations of

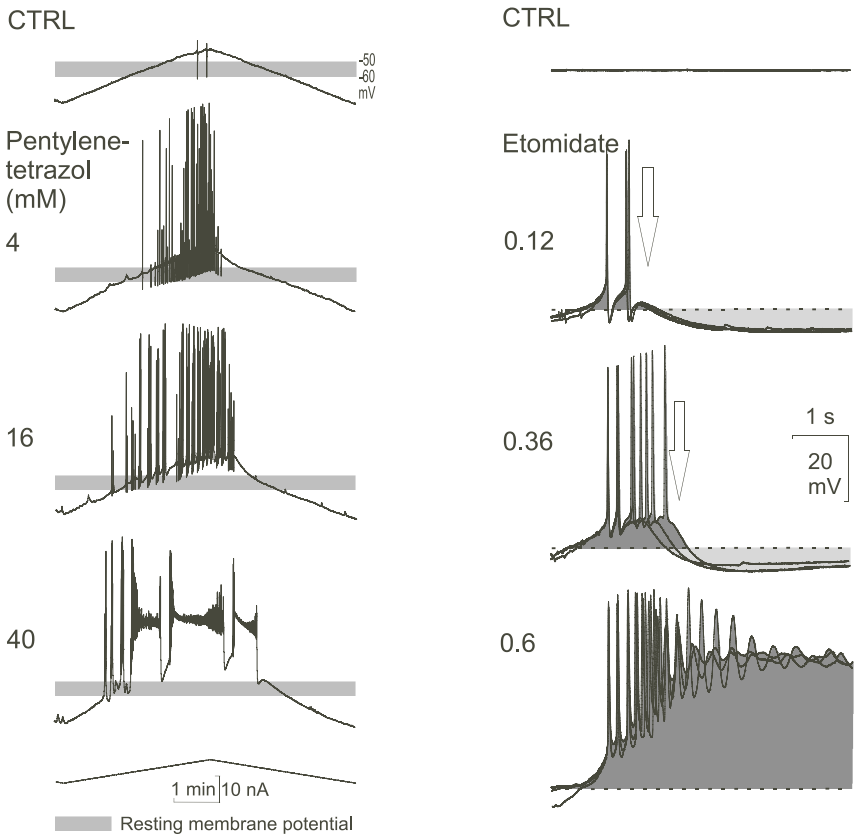

Fig. 4. Pacemaker potentials in neuron B3 during control (CTRL) and "epileptogenic conditions" (pentylenetetrazol: 4 mM, 16 mM, 40 mM; etomidate: 0.12 mM, 0.36 mM, 0.6 mM). Left collumn: A slow triangular current was injected into the neuronal soma, via a second intracellular microelectrode. The level of resting membrane potentials is marked by a grey bar. Oscilloscope recordings. Right collumn: Changes in the shape of pacemaker potentials during increasing concentration of the epileptogenic drug. Pacemaker depolarization and repolarization are marked in dark and light grey, respectively. The begin of repolarization is marked by arrows. Oscilloscope recordings, three signals are superimposed triggered from the first action potential

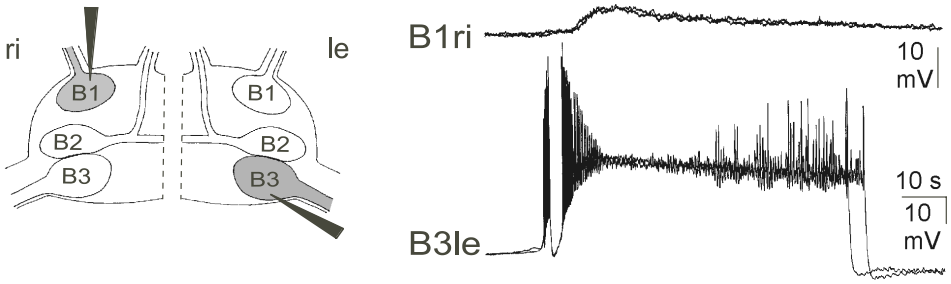

Fig. 5. Simultaneous recording from neurons in the buccal ganglia of *Helix pomatia* during "epileptogenic conditions" application of pentylenetetrazol (40 mM). The schematic drawing shows lateral parts of both buccal ganglia. The impaled somata are marked. Simultaneous recording of neurons B1 right ganglion (ri) and neuron B3 left (le) ganglion. Two recoding are superimposed triggered from the first action potential in neuron B3

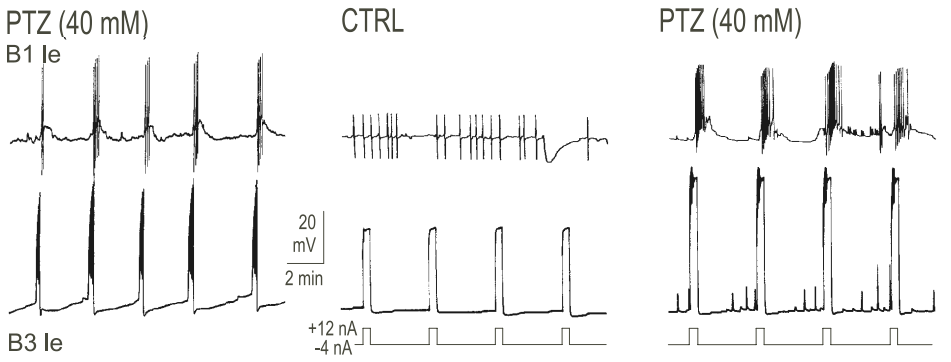

Fig. 6. Simultaneous recordings from neurons B1 and B3 in the left (le) buccal ganglion during control (CTRL) and "epileptogenic conditions" (pentylenetetrazol, PTZ: 40 mM). Pen recordings. In the middle and right pair of recordings, current was injected into the soma of neuron B3 via a second intracellular microelectrode

up to 20 mV which followed the begin of a PDS in neuron B3 (Fig. 5). These PDS-related depolarizations (PDS-RD) showed a number of unique properties: (1) Related depolarizations which followed PDS in neuron B3 appeared in all so far tested buccal neurons. (2) PDS-RD appeared although synaptic potentials were depressed. (3) PDS-RD were blocked in "high Mg-low Ca" solution. (4) PDS-RD were attached to the begin of a PDS in neuron B3, amplitude and duration of PDS appeared to be without effect (cf. Figs 5 and 6, left pair of recordings). (5) PDS-RD exhausted when frequency of PDS was above 1/min. (6) Amplitude of PDS-RD increased during several hours of "epileptic conditions". (7) Several observations demonstrate that the related depolarization is primarily induced from the neighboured neuron B3.

Many of the above listed properties could result from a chemical synaptic contact between neurons B3 and the other buccal neurons. Thus, several series of experiments were done to find such connections [5]. One experiment is shown in Fig. 6. The simultaneous recordings of neurons B3 and B1 on the left side of the Fig. shows PDS in neuron B3 and PDS-RD in neuron B1. In the middle pair of recordings neuron B3 was depolarized under control conditions using pulses to mimic the PDS. The current pulses did not evoke any response in neuron B1. The pulses were again applied under "epileptic conditions" and the related depolarizations (right pair of recordings) re-appeared. Thus, PDS-RD appeared exclusively under "epileptic conditions" and PDS-generating neurons and PDS-RD-neurons were not coupled synaptically. As a whole, the unique properties of PDS-RD lead to the interpretation that synchronization of neurons during "epileptic conditions" results from an extra-synaptic release of substances, e.g. from the dendritic surface of neuron B3 to the dendrites of the other neurons in the buccal ganglion.

DISCUSSION

Pacemaker potentials are found in many neurons and many nervous systems when the neurons were slightly depolarized. A relation between pacemaker potentials and epileptic activity has been suspected several times [12, 15]. They were, however, not regarded as essential basic mechanisms underlying PDS since the essential neuronal synchronization was thought to result from enlarged chemical synaptic EPSP and/or missing IPSP. Although neurons which are able to generate pacemaker potentials may be coupled via electric synapses [13], the degree of electric coupling in the adult nervous system appears to be too low to serve for neuronal synchronization during a seizure. Increases in extracellular potassium concentration has been thought to induce synchronization. The presently observed PDS-RD, however, show properties which are not in line with an increase in $[K^+]_o$ (cf. Figs 5, 6). Presently, non-synaptic release of substances was not shown directly but is an attractive hypothesis. It has been shown previously that vertebrate and invertebrate neurons are able to unspecific exocytosis, provided they are strongly depolarized and intracellular calcium concentration is increased [6, 7, 10, 11, 16]. Both conditions are present when PDS are generated.

Assuming that pacemaker potentials are the basis of neuronal epileptic activity, and that extra-synaptic release of substances is the basis of neuronal synchronization, it is presently not explained what "epileptogenic conditions" are. Since very different means (like e.g. topical application of metals, several types of mechanical lesions, or epileptogenic drugs) can establish these conditions in many nervous systems, it appears probable that the process is unspecific.

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