SYNAPTIC POTENTIALS IN SMOOTH-MUSCLE CELLS

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Tests were carried out on the smooth-muscle cells (SMC) of the anococcygeus muscle of rats and rabbits by the double "sucrose gap" method with Krebs' solution containing tetraethylammonium (1 mmole/liter). Stimulation of a muscle strip from the anococcygeus of rats and rabbits by square pulses of maximal amplitude and short duration induced excitatory postsynaptic potentials (EPSPs) in rat and rabbit SMC and inhibitory postsynaptic potentials (IPSPs) in rabbit SMC. The amplitude of the postsynaptic potentials was a linear function of the membrane potential. Removal of chlorine ions from the external solution reduced the amplitude of EPSPs of SMC of the rabbit anococcygeus and shifted the reversal potential toward the sodium equilibrium potential. EPSP generation in SMC of the rabbit anococcygeus is evidently connected with an increase in membrane permeability to both sodium and chlorine ions.

KEY WORDS: *Smooth muscles; synaptic potentials; role of sodium and chlorine ions.*

Results of microelectrode studies of neuromuscular transmission in the smooth-muscle cells (SMC) of the anococcygeus muscle have recently been published [3-5]. Brief intramural electrical stimulation with maximal amplitude has been shown to induce excitatory postsynaptic potentials (EPSPs) in the SMC of rats and rabbits and inhibitory postsynaptic potentials (IPSPs) in rabbit SMC. The reversal potential has also been found for the EPSPs and an increase in membrane conductance observed during their generation. However, it is not yet known which ions are concerned in the increase in membrane permeability during EPSP development.

The investigation described below was carried out to study this problem.

EXPERIMENTAL METHOD

SMC of the rat and rabbit anococcygeus muscle were the test object. Investigations were carried out on muscle strips 15-20 mm long and 0.2 mm wide by the double "sucrose gap" method [1, 2], using a Berger's chamber [2]. Electrical potentials were derived with Ag–AgCl electrodes. Contact between electrodes and muscle was effected through "agar bridges." The nervous structures of the muscle strip were stimulated with square pulses of maximal amplitude and with a duration of 0.1-0.5 msec. The original Krebs' solution had the following composition (in mmoles/liter double-distilled water): NaCl 133.0, $NaHCO_3$ 16.3, NaH_2PO_4 1.38, KCl 5.0, $CaCl_2$ 2.8, $MgCl_2$ 0.1, glucose 7.8. Krebs' solution with a greatly reduced concentration of chlorine ions was prepared by replacing all the NaCl by sodium methylsulfate or sodium isothionate and all the KCl by potassium methylsulfate or potassium isothionate. The temperature of the test solutions was kept constant at 36°C. Because of the lowered excitability of SMC of the anococcygeus [4] the tests were carried out in the presence of tetraethylammonium (1 mmole/liter).

EXPERIMENTAL RESULTS

Intramural stimulation of a muscle strip from rats in most experiments was accompanied by the development of an EPSP in the anococcygeus SMC. The maximal amplitude of EPSP in the

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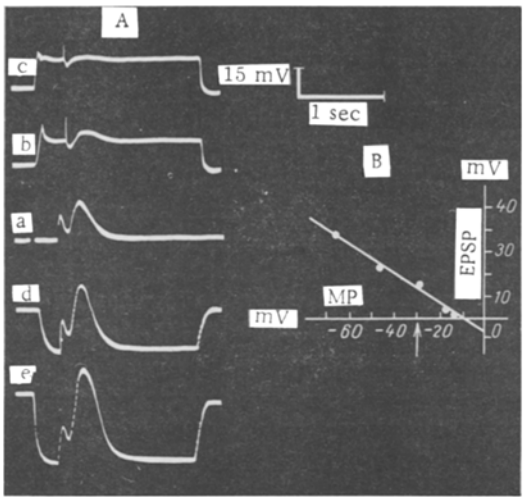

Fig. 1

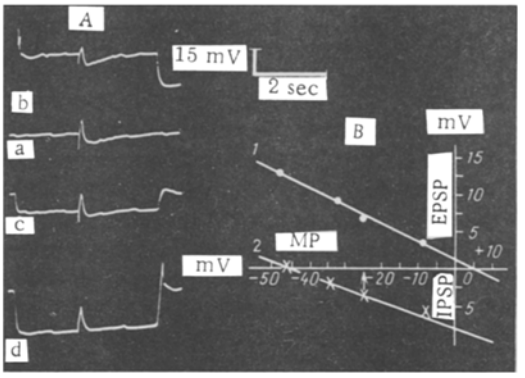

Fig. 2

Fig. 1. Changes in EPSPs of SMC of rat anococcygeus depending on degree of membrane polarization. A) Changes in EPSPs following hyperpolarization and depolarization of SMC membrane: a) normal EPSP; b, c and d, e) after depolarization and hyperpolarization of membrane, respectively. B) Graph of EPSP amplitude as a function of MP amplitude. Arrow on graph points to normal value of MP. On this and subsequent graphs: ordinate, amplitude of synaptic potentials (in mV); abscissa, amplitude of MP (in mV).

Fig. 2. Changes in EPSP and IPSP of SMC of rabbit anococcygeus depending on degree of membrane polarization. A) Changes in EPSP and IPSP during hyperpolarization and depolarization of SMC membrane: a) normal EPSP; b and c, d) during depolarization and hyperpolarization of membrane, respectively. B) graph of amplitude of EPSP and IPSP as functions of MP amplitude. Arrow on graph indicates normal MP. 1) Amplitude of EPSP, 2) of IPSP under normal conditions and during hyperpolarization and depolarization of membrane.

rat SMC was 15 mV and the minimal amplitude 5 mV. The mean amplitude of the EPSPs was 11.4± 0.72 mV (n=20). The membrane potential (MP) varied from 25 to 35 mV with a mean value of 30.5±0.77 mV (n=20). The latent period of onset of the EPSPs varied between 100 and 150 msec, with a mean value of 108±4.2 msec (n=20). The duration of the EPSP from its beginning to its peak level of depolarization [5] was 100-150 msec, with a mean value of 129±5.3 msec (n=20).

The amplitude of the EPSP depended on the degree of membrane polarization. An EPSP in the initial state is illustrated in Fig. 1A, a. After preliminary hyperpolarization of the SMC by the passage of a direct current, intramural stimulation of the same strength and duration led to the appearance of EPSPs of much higher amplitude (Fig. 1A, d, e).

Preliminary depolarization of the rat SMC led to a reduction in the EPSP, and after depolarization of the membrane by 20 mV the EPSPs disappeared almost completely (Fig. 1A, b, c).

Because of the rectifying properties of the SMC membrane of the rat anococcygeus [5], it was impossible to suppress the EPSP completely, for depolarization of more than 20 mV could not be produced even by using very strong currents.

A graph showing the amplitudes of the EPSPs illustrated in Fig. 1A as a function of MP amplitude is given in Fig. 1B. This graph shows that the EPSP amplitude is a linear function of the MP amplitude. The reversal potential was judged from the point where the curve intersected the abscissa. Clearly the reversal potential in this case was —10 mV (Fig. 1B). Besides changes in the amplitude of EPSP depending on the amplitude of MP, a shift in the temporal characteristics of the EPSP also was observed. For instance, after membrane hyperpolarization the rate of rise of the EPSP increased and the duration of its fall was reduced (Fig. 1A, d, e). After depolarization the opposite picture was observed: a decrease in the rate of rise of the EPSP and an increase in the duration of its fall (Fig. 1A, b, c).

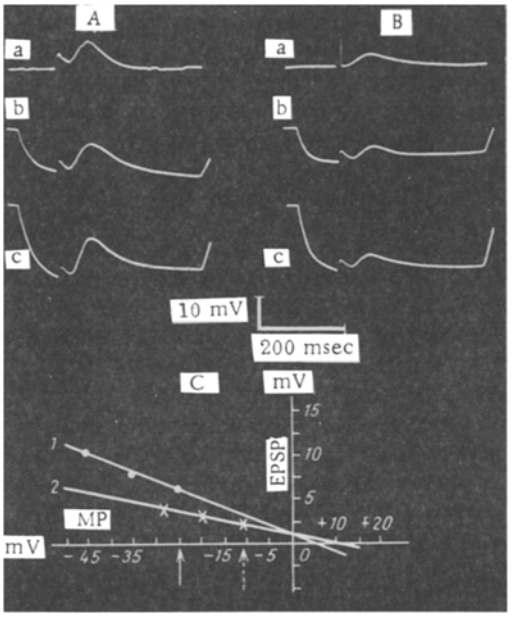

Fig. 3. Effect of removal of chlorine ions from external solution on EPSP of SMC of rabbit anococcygeus. A: a) EPSP under normal conditions; b, c) during hyperpolarization. B: a) EPSP in chloride-free Krebs' solution; b, c) during hyperpolarization in chloride-free Krebs' solution. C) Graph of EPSP amplitude as a function of MP amplitude under normal conditions and after removal of chlorine ions from external solution: 1) amplitude of EPSP under normal conditions, 2) in chloride-free Krebs' solution. Continuous arrow indicates MP of GMC under normal conditions, broken arrow indicates MP of SMC in chloride-free Krebs' solution.

The postsynaptic potential of the SMC of the rabbit anococcygeus is a compound potential consisting of initial depolarization changing into hyperpolarization. In some preparations the depolarization phase predominated (Fig. 3A, a). In muscles in which compound synaptic potentials (Fig. 2A, a) with marked hyperpolarization were observed the amplitude of the EPSP did not exceed 7 mV and its minimal value was 2 mV. The mean amplitude of the EPSPs was 4.8±0.42 mV (n=16) and of the IPSPs 3.3±0.2 mV (n=13). The MP varied from −20 to −25 mV (mean −23±0.81 mV, n=13). The latent period of the EPSP varied from 25 to 40 msec (mean 31.2±1.41 msec, n=16). The duration of the EPSP varied from 30 to 40 msec (mean 31±0.60 msec, n=16), and of the IPSP from 100 to 150 msec (mean 123±3.44 msec, n=13).

Records of one experiment are given in Fig. 2A. As will be clear from Fig. 2A (a), a single stimulus of maximal strength and 0.2 msec in duration evoked an EPSP which changed into an IPSP. A shift of MP toward hyperpolarization led to an increase in the amplitude of the EPSP and a decrease in that of the IPSP (Fig. 2A, c). Further hyperpolarization evoked a still greater increase in EPSP and complete disappearance of the IPSP (Fig. 2A, d). After depolarization of SMC the EPSP was reduced but the IPSP was about doubled in amplitude (Fig. 2A, b). A graph showing the amplitude of the synaptic potentials as a function of MP amplitude is given in Fig. 2. Just as in the experiments on SMC of the rat anococcygeus, the amplitude of the synaptic potential was a linear function of the resting potential. Since the SMC membrane of the rabbit anococcygeus possesses even more marked rectifying properties than the SMC membrane of the rat anococcygeus, depolarization by more than 16 mV was impossible to induce in these experiments. For that reason, just as before, the reversal potential was judged from the point where the IPSP and EPSP curves intersected the abscissa. As Fig. 2B shows, the reversal potential for the EPSP was +5 mV and for the IPSP −45 mV.

In the next series of experiments the effect of a change in the concentration of chlorine ions on EPSP generation by SMC of the rabbit anococcygeus was investigated. A sharp

decrease in the chlorine ion concentration in the Krebs' solution was accompanied by depolarization of the membrane and by the appearance or an increase in frequency of spontaneous spike discharges and a decrease in amplitude of the synaptic potentials. Records of one experiment are given in Fig. 3. Clearly under normal conditions in response to intramural stimulation of the muscle the membrane generated an EPSP. Hyperpolarization of the SMC membrane was accompanied by an increase in the EPSP, the amplitude of which varied as a linear function of the MP amplitude (Fig. 3A, b, c).

Removal of the chlorine ions from the Krebs' solution led within a few minutes to a reduction in the EPSP amplitude. After the action of this solution for 10-13 min the decrease in EPSP was maximal (Fig. 3B, a). Subsequent rinsing of the SMC with chloride-free Krebs' solution did not change the amplitude of the EPSP. Hyperpolarization of the SMC membrane under these conditions, just as normally, caused an increase in EPSP amplitude, but the degree of this increase was much smaller than normally (Fig. 3B, b, c). It must be emphasized that although during hyperpolarization the MP level was actually a little above normal, the amplitude of the EPSP was much smaller than normally (Fig. 3B, b, c). A graph of EPSP amplitude as a function of MP amplitude before and after removal of chlorine ions from the Krebs' solution is shown in Fig. 3C. Clearly the amplitude of the EPSP remained a linear function of MP amplitude even in the chloride-free Krebs' solution. However, in the latter case, the point of intersection of the curve with the abscissa was shifted even further toward the positive side.

Since the reversal potential of the EPSP was closer to the calculated sodium equilibrium potential [5] and since removal of chlorine ions from the Krebs' solution causes only incomplete inhibition of the EPSPs, with a decrease in their amplitude and an even greater shift of the reversal potential toward the positive side, it can be postulated that both chlorine and sodium ions participate in EPSP generation in the SMC of the rabbit anococcygeus muscle.

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