Calcium Mechanism of Norepinephrine Activation of Ionic Pump in Somatic Cells of *Lumbricus Terrestris* Earthworm Muscle Wall

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Blockade of norepinephrine-induced hyperpolarization in earthworm muscle cells was observed in a calcium-free medium, after substitution of Ca^{2+} with Mn^{2+}, and in the presence of verapamil or chlorpromazine to the incubation saline. Changes in Ca^{2+} concentration in the saline and addition of caffeine had no effect on the resting potential of muscle cells. It was hypothesized that signal transduction from norepinephrine-activated membrane adrenoceptors to the ionic pump in earthworm muscle cells depends on influx of extracellular Ca^{2+} and subsequent involvement of Ca^{2+}-accepting proteins similar to calmodulin in vertebrates.

Key Words: *norepinephrine; muscle cells; earthworm*

It was shown that resting membrane potential (RMP) in muscle cells of the earthworm musculocutaneous sac (MCS) is a sum of potassium and chlorine diffusion potentials plus potential created by ionic pumps [3,4]. Norepinephrine hyperpolarizes muscle membrane and increases activity of ionic pumps [3]. This effect develops only in the presence of Ca^{2+} in the incubation medium [5]. However, calcium mechanism of norepinephrine-induced activation of ionic pump remains unclear, which determined the purpose of the study.

MATERIALS AND METHODS

Experiments (n=79) were carried out on superficial fibers of longitudinal muscle bundles on the inner MCS surface from *Lumbricus terrestris* earthworms. Freshly isolated MCS preparations free from coelomic organs (10-15 segments in length) were placed in a bath for electrophysiological studies filled with modified Drewes-Pax solution [3,9]. The medium contained (in mmol/liter): 163 Na^+, 4 K^+, 6 Ca^{2+}, 93 Cl^-, 43 SO_4^{2-}, 2 $Tris^+$, and 167 sucrose (osmolarity 478 mosmol/liter, ionic strength 229 mmol/liter, pH 7.2-7.4) at room temperature. To maintain osmolarity in solutions with increased (12 mmol/liter) or decreased (0 and 3 mmol/liter) Ca^{2+} concentration, Na^+ content was proportionally decreased (151 mmol/liter) or increased (175 and 169 mmol/liter, respectively). In some experiments Ca^{2+} was substituted with 6 mmol/liter Mn^{2+}.

RMP in muscle fibers was measured using glass microelectrodes filled with 2.5 M KCl (7-15 MΩ tip resistance) and connected to a standard recorder. RMP was recorded before and 10-15 min after changing solutions or addition of test agents. Norepinephrine (10^{-5} mol/liter, Sigma), verapamil hydrochloride (10^{-4} mol/liter), caffeine (10^{-3} mol/liter, Serva) and chlorpromazine (10^{-5} mol/liter, Sigma) were used.

RESULTS

The presence of norepinephrine in the incubation solution increased RMP in earthworm muscle cells (Table 1). Oubain, α- and β-adrenoblockers abolished this

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effect of norepinephrine [3-5]. It was concluded that norepinephrine-induced hyperpolarization depended on activation of ionic pumps and membrane structures similar to α- and β-adrenoceptors in vertebrates [5]. RMP of muscle cells decreased in a Ca^{2+}-free solution (Table 1). Similar depolarization developedafter inactivation of ionic pumps with oubain [3,5]. Hence ionic pump works only in the presence of extracellular Ca^{2+} [5]. RMP of muscle cells remained unchanged in the presence of 2-fold lower and 2-fold higher Ca^{2+} concentrations (Table 1). It is possible that RMP and activity of ionic pump do not depend directly on the concentration of extracellular Ca^{2+}, but only on the presence of Ca^{2+} ions. This suggests that Ca^{2+} acts a messenger in activation of ionic pump. Norepinephrine did not increase RMP in a Ca^{2+}-free medium (Table 1), but even decreased it ($p<0.01$, Table 1). Calcium channel blocker verapamil [1,8] did not change RMP (Table 1), but inhibited hyperpolarizing effect of norepinephrine and induced membrane depolarization in a calcium-free medium ($p<0.001$, Table 1). Substitution of Ca^{2+} with calcium channel inhibitor Mn^{2+} [6] decreased RMP, while addition of norepinephrine to the Mn^{2+}-containing solution promoted RMP decrease ($p<0.001$, Table 1) similar to the effect of verapamil.

This phenomenon cannot be unambiguously interpreted on the basis of the available data and requires further studies. It is known that caffeine mobilizes calcium from intracellular depots [11] and increases its cytoplasmic concentration. However, in our experiments, caffeine produced no effect on RMP of muscle cells. It is possible that earthworm MCS cells contain no intracellular calcium stores (similar to sarcoplasmic reticulum of skeletal muscles). Caffeine acts as a nonspecific inhibitor of cAMP- and cGMP-dependent phosphodiesterases [7] and increases intracellular concentration of cyclic nucleotides. However our previous studies showed that the increase in intracellular cAMP and cGMP concentrations [2] did not change resting potential in earthworm somatic muscle cells [5]. Thus, experiments with caffeine supported previous hypothesis that cAMP and cGMP are not involved in the mechanisms of signal transduction from adrenoceptor structures of the muscle cell membrane to ion transporting systems. Experiments with caffeine and calcium channel blockers showed that realization of northe effect of epinephrine on ionic pumps requires extracellular Ca^{2+}, but not its mobilization from intracellular stores. Ca^{2+} not only activates the intracellular cyclic nucleotide system [7], but acts via other calcium acceptor systems (*e.g.* calmodulin) [10]. In experiments with norepinephrine, calmodulin blocker chlorpromazine [10] inhibited norepinephrine-induced hyperpolarization of muscle cell membrane, but had no effect on RMP (Table 1).

TABLE 1. Effect of Norepinephrine, Verapamil, Caffeine, Chlorpromazine, Changes in Extracellular Ca^{2+} Concentration, and its Substitution with Mn^{2+} on RMP in Longitudinal Muscle Bundle of Earthworm *Lumbricus terrestris* (*M*±*m*)

Experimental conditions	RMP, mV
Control (*n*=400)	48.7±0.6
Norepinephrine (*n*=100)	55.8±1.2
Ca^{2+}, mmol/liter 0 (*n*=120)	41.5±0.9*
3 (*n*=121)	47.7±0.7
12 (*n*=121)	49.1±0.9
Ca^{2+}, 0 mmol/liter+norepinephrine (*n*=120)	35.5±0.5
Verapamil (*n*=121)	48.6±0.8
Verapamil+norepinephrine (*n*=120)	42.9±0.8*
Mn^{2+}, 6 mmol/liter (*n*=114)	44.3±1.0**
+norepinephrine (*n*=120)	38.5±0.7*
Chlorpromazine (*n*=120)	48.0±1.0
+norepinephrine (*n*=120)	50.2±0.9
Caffeine (*n*=124)	50.5±0.9

Note. *$p<0.001$, **$p<0.01$ compared to the control (normal saline).

Thus, norepinephrine binding to adrenoceptors on the plasma membrane of earthworm muscle cells induces Ca^{2+} influx. Ca^{2+} ions do not activate the cyclic nucleotide system, but activate membrane ionic pumps via some calcium acceptor proteins similar to calmodulin in vertebrates. This, in turn, increases the contribution of electrogenic ionic pumps into the integral RMP and leads to membrane hyperpolarization in the earthworm muscle cells.

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