# Cholinergic and GABAergic Innervation Regulate Activity of Electrogenic Ionic Pump in Earthworm Somatic Muscle Cells

**E. M. Volkov, A. R. Sabirova, M. E. Volkov, and A. L. Zefirov**

Translated from *Byulleten' Eksperimental'noi Biologii i Meditsiny*, Vol. 144, No. 8, pp. 128-130, August, 2007
Original article submitted November 27, 2006

Carbacholine reduced, while baclofen and norepinephrine increased resting membrane potential in earthworm somatic muscle cells. In the presence of carbacholine, neither norepinephrine, nor baclofen hyperpolarized the membrane. Ouabain decreased resting potential and abolished the effects of carbacholine, norepinephrine, and baclofen on membrane potential. It was hypothesized that carbacholine directly inhibited the ouabain-sensitive component of $Na^+/K^+$ pump and abolished the activating effect of norepinephrine and baclofen.

**Key Words:** *resting potential; $Na^+/K^+$ pump; carbacholine; norepinephrine; baclofen*

Somatic muscle cells of *Lumbricus terrestris* earthworm are characterized by multiterminal and polyneural innervation [8]. It is assumed that acetylcholine and GABA are depolarizing and hyperpolarizing transmitters, respectively [8]. We previously showed that resting membrane potential (RMP) is an integral value; electrogenic ion pumps modulated by both acetylcholine and GABA [5] make a considerable contribution into RMP.

We studied the interaction of choline- and GABAergic systems during regulation of RMP in earthworm somatic muscle cells.

## MATERIALS AND METHODS

Experiments were carried out on surface muscle cells of the inner longitudinal bundles of the musculocutaneous sack of earthworm *Lumbricus terrestris* during the winter period. Freshly isolated and longitudinally cut preparations of musculocutaneous sacks including 10-15 segments free from coelomic organs were placed into a bath for electrophysiological examination with the following solution (in mM): 163.0 $Na^+$; 4.0 $K^+$; 6.0 $Ca^{2+}$; 93.0 $Cl^-$; 43.0 $SO_4^{2-}$; 2.0 $Tris^+$; 167.0 sucrose; osmolarity 478.0 mosmol/liter, ionic strength 229.0 mmol/liter; pH 7.3-7.4 at room temperature [5].

During the first series, baseline parameters were determined, the experimental measurements were made 10-15 min after application of the test substances. RMP was measured with glass microelectrodes filled with 2.4 mM KCl (10-15 MΩ tip resistance) connected to a routine electrophysiological amplifier. Carbacholine (CCh, $1\times10^{-5}$ M), baclofen ($1\times10^{-4}$ M), norepinephrine ($1\times10^{-5}$ M), ouabain ($1\times10^{-4}$ M), atropine ($1\times10^{-5}$ M), and d-tubocurarine were used. All chemicals were from Sigma.

## RESULTS

We previously demonstrated that CCh, similarly to Ach, decreases RMP of earthworm somatic muscle cells in a dose-dependent manner [1,6]. The threshold concentration of CCh not producing muscle contracture was $1\times10^{-5}$ M [6]. In the present ex-

Kazan State Medical University, Ministry of Health of the Russian Federation. ***Address for correspondence:*** emvolkov@kzn.ru. E. M. Volkov

0007-4888/07/1442-0163 

periments, we chose this concentration of CCh, which could depolarize the membrane to a level comparable to that observed during inactivation of ionic pumps by ouabain (Table 1).

Baclofen hyperpolarizes muscle membrane (MM) even more effectively than GABA [7], and norepinephrine was more potent than epinephrine [4]. Thus, among GABA- and adrenergic agents, baclofen and norepinephrine most significantly elevated RMP of muscle cells. While each of these chemicals has its own specific receptor input [4,7], the final result of their action is activation $Na^+,K^+$-ATPase. This activation elevates contribution of the "pump" component into the integral value of RMP in earthworm somatic muscle cells [5], *i.e.* increased transmembrane potential.

In the presence of CCh, baclofen did not hyperpolarize MM, so RMP was lower than in intact muscle (Table 1). Individual application of atropine or d-tubocurarine had no effect on RMP of muscle cells and did not prevent the CCh-induced decrease in RMP of muscle cells (Table 1). In CCh-depolarized cells, baclofen did not increase RMP in the presence of either atropine or d-tubocurarine. It can be hypothesized that CCh blocked the effect of baclofen on $Na^+,K^+$-ATPase, while atropine or curare did not prevent this blockade. The observed phenomenon can be explained by two putative mechanisms: competition at the receptor level (which is hardly possible in view of experiments with muscarinic and nicotinic cholinoceptor blockers) or direct interaction with the ion transporter molecule.

For better understanding of the mechanism of anti-baclofen action of CCh we carried out experiments with norepinephrine. In the presence of CCh, norepinephrine lost its potency to affect RMP, while MM was depolarized relative to the control (Table 1). Thus, baclofen and norepinephrine acting via different specific receptor inputs could not hyperpolarize the membrane in the presence of CCh. Moreover, MM remained depolarized under these conditions. These data suggest that CCh blocks the electrogenic ion pump in the membrane of earthworm muscle cells.

To test this hypothesis, experiments with inactivation of ionic pump with ouabain were carried out. Individual or combined application of ouabain and CCh produced virtually equal depolarization of MM (Table 1). In other words, CCh could not produce extra depolarization of the membrane with inactivated ionic pump. Similar data were obtained in experiments with combined application of ouabain with baclofen or norepinephrine, where RMP decreased and did not differ from RMP in the above experiments CCh and ouabain. These data can be explained by the common mechanism based on inactivation of ionic pump or the loss of its electrogenicity.

Thus, CCh depolarizes earthworm MM. The classical wide-spectrum muscarinic and nicotinic cholinoceptor blockers atropine and d-tubocurarine, as well as specific cholinolytic α-bungarotoxin [6] do not prevent this effect of CCh on RMP [3,6]. As expected, ouabain depolarized the earthworm MM. Both baclofen, specific agonist of GABAergic B-type receptors, or norepinephrine exerting its effect via β-adrenoreceptors [4] loose their potency to hyperpolarize membrane in the presence of ouabain or CCh, so the membrane remains depolarized, and this effect of cholinomimetic CCh cannot be prevented by cholinolytic atropine or d-tubocurarine. CCh produced no extra depolarization in addition to that caused by ouabain, which inhibited electrogenic ionic pump. These findings can be explained by the hypothesis that CCh directly blocks electrogenic ionic pumps in MM, at least, its ouabain-sensitive part. This hypothesis explains inability of atropine or d-tubocurarine to prevent the effect of CCh. Therefore, depolarization of MM by relatively low CCh concentrations results mostly from the loss of pump contribution into the integral RMP of earthworm somatic muscle cell membrane. However, it

**TABLE 1**. Effects of CCh, Baclofen, Norepinephrine, Ouabain, Atropine, and d-Tubocurarine on RMP in Earthworm Somatic Muscle Cells ($M \pm m$)

| Chemicals | RMP, mV |
|---|---|
| Control | 49.0±0.6 |
| CCh | 42.0±1.1* |
| Ouabain | 42.0±0.8* |
| Baclofen | 61.0±0.9** |
| Norepinephrine | 56.0±0.9** |
| Ouabain+baclofen | 42.0±1.0* |
| Ouabain+norepinephrine | 40.0±1.0* |
| CCh+baclofen | 44.0±1.1* |
| Atropine | 47.0±1.1 |
| CCh+atropine | 41.0±1.2** |
| CCh+atropine+baclofen | 43.0±1.1* |
| d-Tubocurarine | 48.0±0.9 |
| CCh+d-tubocurarine | 44.0±1.1* |
| CCh+d-tubocurarine+baclofen | 46.0±1.3* |
| CCh+norepinephrine | 44.0±0.9* |
| CCh+ouabain | 41.0±0.9** |

**Note.** Each series of experiments consisted of 80 measurements made on muscle preparations from 4 animals. *$p<0.01$, **$p<0.001$ compared to the control.

does not exclude the interaction of CCh with acetylcholine-sensitive channel-receptor complexes [2,3].

This work was supported by the Russian Foundation for Basic Research, grant No. 06-04-48458.

## REFERENCES

1. E. M. Volkov and L. F. Nurullin, *Byull. Eksp. Biol. Med.*, **134**, No. 11, 498-500 (2002).
2. E. M. Volkov and L. F. Nurullin, *Ibid.*, **136**, No. 11, 567-570 (2003).
3. E. M. Volkov and L. F. Nurullin, *Ibid.*, **139**, No. 3, 392-396 (2005).
4. E. M. Volkov, L. F. Nurullin, G. I. Blokhina, and A. A. Nikol'skii, *Ibid.,* **132**, No. 9, 244-246 (2001).
5. E. M. Volkov, L. F. Nurullin, and A. A. Nikol'skii, *Ros. Fiziol. Zh.*, **87**, No. 3, 329-334 (2001).
6. E. M. Volkov, L. F. Nurullin, and V. N. Frosin, *Byull. Eksp. Biol. Med.*, **132**, No. 4, 469-471 (2001).
7. E. M. Volkov, A. R. Sabirova, S. N. Grishin, and A. L. Zefirov, *Ibid.*, **139**, No. 2, 219-223 (2005).
8. O. F. David, *Morphological Principles of Annelid Locomotion* [in Russian], Leningrad (1990).