# EXPERIMENTAL BIOLOGY

# Mechanisms of Hyperpolarizing Effect of GABA on Resting Potential of the *Lumbricus Terrestris* Muscular Wall Somatic Cells

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GABA, baclofen, isoguvacine increase, and cis-4-aminocrotonic acid does not modify resting membrane potential of muscle cells. Bicuculline, phaclofen, N-ethylmaleimide, chlorpromazine, verapamil, and removal of $Ca^{2+}$ from bathing solution abolished the effect of baclofen, while U73122 and D609 were ineffective in this respect. The authors conclude that the *Lumbricus terrestris* muscle cells contain GABAergic structures similar to a- and b-receptors. Activation of GABA receptors induced $Cl^-$ inward current and $Ca^{2+}$ entry with subsequent activation of calmodulin-like proteins, which causes membrane hyperpolarization by increasing the effect of "pumping potential" on resting membrane potential.

**Key Words:** *GABA; resting potential; $Ca^{2+}$; muscle cells; Lumbricus terrestris*

Somatic muscle cells of *Lumbricus terrestris* musculocutaneous sac wall are sensitive to GABA [4]. We previously showed that the hyperpolarizing effect of GABA on the membrane is caused primarily by stimulation of active amperogenic ionic pump [1]. The pharmacological type of sarcolemmal GABAergic receptors and the mechanisms of the stimulatory signal transfer from these receptors to the ion-transporting proteins of the *Lumbricus terrestris* muscle cell membranes remain unclear. The study of these mechanisms became the object of our research.

## MATERIALS AND METHODS

Experiments were carried out on surface muscle cells of longitudinal bundles of the inner side of the *Lumbricus terrestris* musculo-cutaneous sac. Fresh preparations of longitudinally dissected fragments (10-15 segments long) free from celomic organs were placed in a cuvette for electrophysiological studies with modified Dreves—Pax solution [3] (mmol/liter): 163 $Na^+$; 4 $K^+$; 6 $Ca^{2+}$; 93 $Cl^-$; 43 $SO_4^{2-}$; 2 Tris; 167 sucrose; osmolarity 478 mosmol/liter, ionic strength 229 mmol/liter, pH 7.2-7.4 at room temperature. The muscle cell resting membrane potential (RMP) was measured with glass microelectrodes filled with 2.5 mol/liter KCl with 10-15 mΩ tip resistance. RMP measurements were carried out before and after addition of drugs. The following drugs were used: $10^{-4}$ mol/liter GABA; $10^{-4}$ mol/liter isoguvacine; $10^{-4}$ mol/liter baclofen; $10^{-4}$ mol/liter cis-4-aminocrotonic acid; $5\times10^{-4}$ mol/liter bicuculline; $10^{-4}$ mol/liter phaclofen (all from Sigma); $10^{-4}$ mol/liter verapamil (Russia); $10^{-4}$ mol/liter chlorpromazine (Serva); $10^{-4}$ mol/liter N-ethylmaleimide (Sigma); $10^{-4}$ mol/liter U73122 (Sigma); and $10^{-4}$ mol/liter D609 (Sigma).

## RESULTS

Addition of GABA into bathing solution increased RMP of muscle cells (Table 1). The increment in trans-

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membrane potential difference was significant (about 20% of the initial value, according to our data). The effect of GABA on muscle cells is realized through activation of the GABAergic receptor family, including a-, b-, and c-receptors [5]. Isoguvacine, a selective a-receptor agonist [6], hyperpolarized muscle membrane (Table 1), but this effect was less pronounced compared to that of GABA (10 *vs.* 20% relative increase in RMP). Cis-4-aminocrotonic acid, a selective c-receptor agonist [6], was ineffective as a modifier of the *Lumbricus terrestris* muscle cell RMP (Table 1). On the other hand, addition of b-receptor agonist baclofen [5,6] to the bathing solution caused the most potent increase in RMP (Table 1): almost 25% of the initial value. From experiments with GABAergic receptor agonists an idiogram was plotted based on the criterion of efficiency of the test agents in increasing the transmembrane potential difference in *Lumbricus terrestris* muscle cells. Cis-4-aminocrotonic acid did not modify RMP. The *Lumbricus terrestris* muscle cell membrane was most of all sensitive to baclofen (b-receptor activator), least of all to isoguvacine (a-receptor selective agonist), and insensitive to cis-4-aminocrotonic acid (c-receptors). Hence, the *Lumbricus terrestris* muscle membrane contains a- and b-receptors, but not c-receptors. Another interpretation is possible: the sarcolemma contains a universal GABAergic receptor structure possessing the highest affinity for baclofen, weaker for isoguvacine, and is insensitive to cis-4-aminocrotonic acid. This is true only for the structures similar by their pharmacological characteristics to, primarily, b-receptors and less so to a-receptors.

Further analysis of selective activity of postsynaptic GABAergic sensitivity of muscle cells was carried out using the corresponding antagonists. Addition of bicuculline (a nonspecific inhibitor of sensitivity to GABAergic preparations) depolarized muscle membrane. However, phaclofen (specific b-receptor inhibitor) did not modify RMP (Table 1). The potential created by the work of active ionic transporting systems plays an important role in the integral RMP of *Lumbricus terrestris* muscle cells [2]. Membrane-hyperpolarizing effect of GABA is due to activation of amperogenic ionic pumps [1]. It is also known, that *Lumbricus terrestris* somatic muscle cells possess double innervation: stimulating (depolarizing) with acetylcholine mediator and inhibitory (hyperpolarizing) with GABA mediator [4]. Presumably, the depolarizing effect of bicuculline on muscle cell RMP is determined by inhibition of GABAergic innervation. However, the fact that phaclofen has no effect on RMP attests to possible direct effect of bicuculline on the mechanisms responsible for generation of the resting potential or to inability of phaclofen to completely abolish the effect of GABAergic innervation. Isoguvacine and baclofen did not modify muscle cell RMP in the presence of bicuculline. Baclofen in combination with phaclofen also failed to hyperpolarize the muscle membrane (Table 1). Hence, GABAergic structures of the postsynaptic membrane of *Lumbricus terrestris* muscle cells by their pharmacological properties are most close to b-receptors of vertebrate cells, or, which is more probable, possess both a- and b-receptors, the latter population predominating in this cell phenotype.

$Cl^-$ plays an important role in the generation of the *Lumbricus terrestris* muscle cell RMP [2,11]. a-Receptors are conjugated with Cl-channels, GABA activation of these channels provides $Cl^-$ inward current causing membrane hyperpolarization [5]. Therefore, modification of the membrane chlorine conduction can be one of the mechanisms of GABA effect on RMP. However, activation of the Na/K pump and chlorine co-transport play a more important role in RMP increment [10]. b-Receptors (predominant type according to our data) are coupled through G proteins with, among other things, membrane calcium channels [5]. $Ca^{2+}$ plays the key role in signal transduction from receptors to the ion-transporting systems [3]. In our experiments baclofen in a calcium-free solution was ineffective (Table 1), which confirms this hypothesis. Verapamil ($Ca^{2+}$-channel blocker) also prevents baclofen effect on RMP (Table 1). These data suggest that $Ca^{2+}$ entry is a mechanism triggering activation of ionic pumps. Inward $Ca^{2+}$ current is caused by activation of GABAergic receptors of presumably b-type. G proteins are an obligatory link between GABA b-receptors and $Ca^{2+}$ channels [5]. The presence of N-ethylmaleimide (destructor of G proteins [9]) in the medium depolarizes muscle membrane. Baclofen in the presence of N-ethylmaleimide is unable to modify RMP (RMP in this case is reduced, Table 1). Experiments with N-ethylmaleimide, similarly as with bicuculline, confirmed the hypothesis on the important role of GABAergic innervation in the maintenance of RMP in *Lumbricus terrestris* muscle cells. Individual application of U73122 (PI phospholipase C blocker [8]), D609 (PC phospholipase C blocker [7]), chlorpromazine (calmodulin inhibitor [6]) did not modulate RMP in muscle fibers. The presence of U73122 or D609 in the solution did not abolish hyperpolarization, while chlorpromazine reduced the effect of baclofen on muscle cell RMP (Table 1). The increase of intracellular concentration of cAMP or cGMP did not increase RMP of muscle cells [3]. Hence, adenylate and guanylate cyclase systems and pathways accompanied by production of ionisitoltriphosphate and diacylglycerol are not involved in the processes of intracellular signaling from GABAergic receptors to ionic pumps,

**TABLE 1.** Effects of GABA, Baclofen, Bicuculline, Verapamil, Isoguvacine, Phaclofen, Chlorpromazine, $Ca^{2+}$-Free Medium, Cis-4-Aminocrotonic Acid, D609, N-ethylmaleimide, and U73122 on RMP of *Lumbricus terrestris* Somatic Muscle Cells (*M*±*m*)

| Experimental conditions | RMP, mV | Cell number |
|---|---|---|
| Control | 48.7±0.6 | 400 |
| GABA, $1\times10^{-4}$ mol/liter | 58.1±0.8* | 240 |
| Baclofen, $1\times10^{-4}$ mol/liter | 61.0±0.9* | 160 |
| Isoguvacine, $1\times10^{-4}$ mol/liter | 52.4±0.9* | 160 |
| Cis-4-aminocrotonic acid, $1\times10^{-4}$ mol/liter | 51.3±1.1 | 160 |
| Bicuculline, $5\times10^{-4}$ mol/liter | 42.5±1.0* | 160 |
| Bicuculline, $5\times10^{-4}$ mol/liter+baclofen, $1\times10^{-4}$ mol/liter | 49.2±1.0 | 120 |
| Bicuculline, $5\times10^{-4}$ mol/liter+isoguvacine, $1\times10^{-4}$ mol/liter | 46.3±1.1 | 120 |
| Phaclofen, $1\times10^{-4}$ mol/liter | 48.6±1.2 | 160 |
| Baclofen, $1\times10^{-4}$ mol/liter+phaclofen, $1\times10^{-4}$ mol/liter | 47.1±1.1 | 160 |
| Chlorpromazine, $1\times10^{-4}$ mol/liter | 48.0±0.9 | 120 |
| N-ethylmaleimide, $1\times10^{-4}$ mol/liter | 43.0±1.2* | 120 |
| U73122, $1\times10^{-4}$ mol/liter | 51.1±1.0 | 120 |
| D609, $1\times10^{-4}$ mol/liter | 49.2±1.2 | 120 |
| Baclofen, $1\times10^{-4}$ mol/liter in $Ca^{2+}$-free medium | 49.8±1.0 | 120 |
| Baclofen, $1\times10^{-4}$ mol/liter+verapamil, $1\times10^{-4}$ mol/liter | 48.5±1.2 | 120 |
| Baclofen, $1\times10^{-4}$ mol/liter+chlorpromasine, $1\times10^{-4}$ mol/liter | 49.1±1.1 | 120 |
| Baclofen, $1\times10^{-4}$ mol/liter+N-ethylmaleimide, $1\times10^{-4}$ mol/liter | 44.1±1.1* | 120 |
| Baclofen, $1\times10^{-4}$ mol/liter+D609, $1\times10^{-4}$ mol/liter | 60.5±0.9* | 120 |
| Baclofen, $1\times10^{-4}$ mol/liter+U73122, $1\times10^{-4}$ mol/liter | 59.9±0.9* | 120 |

**Note.** *Significant difference from the control (control: RMP values in standard solution).

while calmodulin or Ca-acceptor proteins play an important role in this process.

Hence, the postsynaptic membrane of *Lumbricus terrestris* somatic muscle cell contains GABAergic structures similar to a- and b-receptors, with predominance of the latter type. Activation of b-receptors through G proteins triggers $Ca^{2+}$ entry from extracellular space into cell cytoplasm with subsequent involvement of calmodulin or similar proteins in the process providing activation of amperogenic ionic pumps, which manifests in the increase in transmembrane potential difference. This mechanism does not rule out the possibility of RMP increase under the effect of a-receptor activation providing chlorine inward current. We can assert that *Lumbricus terrestris* muscle cell RMP is largely regulated by GABAergic innervation.

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