Inhibitory Effect of Clonidine on Cholinergic Transmission

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Equilibrium geometry and electronic structure of three local anesthetic molecules and clonidine were computed. It was shown that clonidine molecule could incorporate into local anesthetic binding centers in potential-operated Na^+ channels and in ionic channels coupled to nicotinic cholinergic receptors. The modulatory effect of clonidine on contractile responses of skeletal muscle showed that blockade of Na^+ channels and nicotinic cholinergic receptors is a part of its analgesic action.

Key Words: *clonidine; analgesia; quantum chemistry computations; synaptic transmission; cholinoreceptor*

We previously showed that α_2-adrenergic receptor agonist clonidine directly inhibits potential-operated Na^+ channels [1]. It was hypothesized that this property contributed to the analgesic action of clonidine. By the range of effective concentrations (20-1000 μM) and the character of its effect on the parameters of Na^+ channels, clonidine is similar to the most efficient local anesthetics (LA) [6]. Considering this feature, we used MINDO3 method to compute equilibrium geometry and electronic structure of clonidine molecule and molecules of three typical LA: 2-(ethylpropylamino)-2',6'-butyroxylidide (etidocaine), procaine, and tetracaine. A certain similarity in the spatial structure of the examined molecules was revealed.

In addition to the blocking action on the potential-operated ionic channels, many LA modify firing of neurons and skeletal and cardiac muscle cells by inhibiting the nicotinic receptor-channel complex [5,6]. This inhibitory effect (IC_{50}) varied form 1 to 70 μM [9]. Some papers showed that clonidine interacts with the cholinergic receptors on chromaffin cells [10,14]. It is also known that many LA directly block Ca^{2+}-releasing channels in the sarcoplasmic reticulum [2, 15]. In this study, we examined the effect of clonidine on the contractile responses of frog skeletal muscle. In this object, contraction can be initiated by several ways involving different links of electromechanical coupling. Moreover, catecholamines modify the contractile responses in cold-blooded animals via β-adrenoceptors [3,8], which suggests that not all effects of clonidine are mediated via α_2-adrenoceptors.

Our aim was to compare structural and electronic geometry of clonidine and typical LA and to examine the effect of this drug on skeletal muscle contraction for identification of specific components of analgesic effect of clonidine.

MATERIALS AND METHODS

Computations of equilibrium geometry and electronic structure of clonidine and three LA molecules (etidocaine, procaine, and tetracaine) were carried out basing on semiempirical MINDO3 method realized in GAUSSIAN 94W software [7].

The effect of clonidine on contractile activity of skeletal muscles was studied on *extensor digitorum longus* IV muscles of *Rana temporaria*. The contractile responses were induced by short (1-2 msec) electrical stimuli (single contractions, SC), by rising K^+ concentration in the bathing solution to 120 mM (po-

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tassium contractions), and by application of exogenous acetylcholine (10^{-5}-10^{-3} M, acetylcholine contractions) or caffeine (1-20 mM, caffeine contractions). The contractions were recorded in isometric mode [2].

The muscle preparation was bathed in Ringer solution containing (in mM): 120 NaCl, 2.5 KCl, 2 $CaCl_2$, 10 HEPES, pH 7.3. All chemicals were from Sigma. The Hill equation was used to calculate IC_{50} for clonidine. The data were averaged (n=6-10).

RESULTS

The computations of equilibrium geometry and electronic structure of clonidine, etidocaine, procaine, and tetracaine revealed similarity in the spatial structure of these molecules (Fig. 1). The molecules differed in hydrophobic and hydrophilic fragments attached to the aromatic ring of LA and clonidine, respectively. The linear size of LA molecule from the carbon atom of the aromatic ring and the most distant backbone atom (carbon or nitrogen) is about 9 and 10 Å for etidocaine and procaine, respectively. In the clonidine molecule, the corresponding distance is 8 Å. In tetracaine molecule, the maximum distance between two carbon atoms situated at the opposite sides from the aromatic ring is 15 Å. In etidocaine molecule, the maximum distance between hydrogen atoms of two methyl groups attached to the ring is 6.5 Å. In procaine and tetracaine molecules, the distance between the hydrogen atoms in aromatic ring is 5 Å. In clonidine molecule, the distance between the chlorine atoms attached to the aromatic ring is 5.5 Å.

The method of site-directed mutagenesis [13] showed that the pore of Na^+ channels contains LA receptor in the transmembrane segment S6 of domain IV in α-subunit [4]. The aromatic ring of the one end of LA molecule binds to Y1771 via π-electron interaction, while the hydrophobic fragment of the other end of this molecule binds to F1764 due to hydrophobic interaction. According to the above computations, the distance between Y1771 and F1764 in the channel pore is about 9-11 Å, which makes possible clonidine incorporation into the site of LA binding in Na^+-channel pore. In contrast to LA molecules, which have two binding sites, clonidine binds to Y1771 of the channel pore only via π-electron interaction of its aromatic ring.

In this study, we examined the effect of clonidine on parameters of contracture response induced by extracellular caffeine (5-10 mM). It was shown that clonidine produced no effect on the amplitude and kinetics of caffeine contracture even at the maximum tested concentrations (Fig. 2, *a*). Since caffeine stimulates Ca^{2+} release from the sarcoplasmic reticulum due to direct effect on the ryanodyne receptors, our data indicate the absence of a direct effect of clonidine on the contractile apparatus and calcium-releasing system. The parameters of potassium contraction varied slightly only at maximum clonidine concentrations (800–1000 μM). The significant decrease of SC amplitude was observed at concentrations exceeding 200 μM (Fig. 2, *b*). Since initiation of SC is mediated by generation of Na^+ action potential in the plasmalemma, the effect of clonidine on SC can serve as a criterion of its blocking action on sodium channels (Fig. 2, *d*).

The concentration of clonidine inducing 2-fold decrease in the amplitude of SC (IC_{50}) was 500-600 μM. The phasic skeletal muscle fibers are characterized by

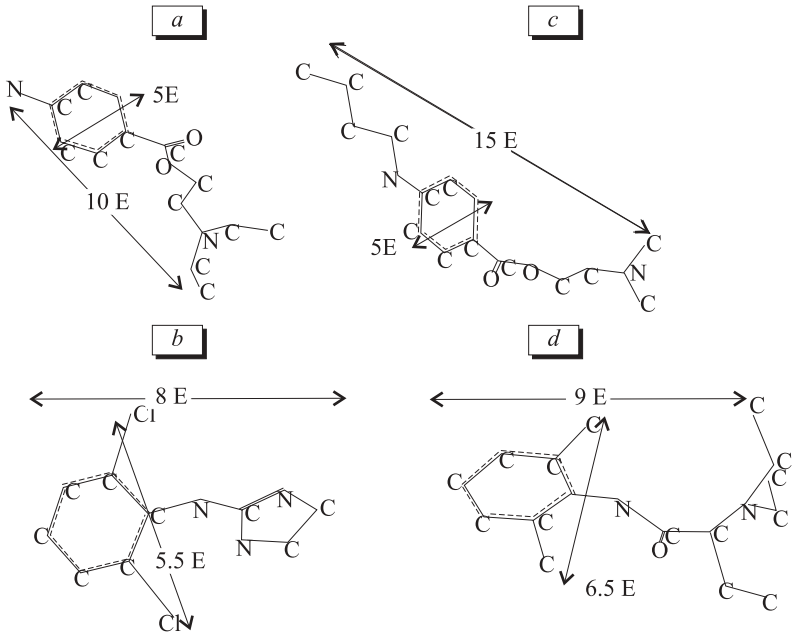

Fig. 1. Spatial structure of procaine (*a*), clonidine (*b*), tetracaine(*c*), and etidocaine (*d*) according to MINDO3 computations. Hydrogen atoms are not shown. The numbers show maximum linear size of the molecules between the most distant carbon and nitrogen atoms and between H(Cl) atoms attached to the aromatic ring.

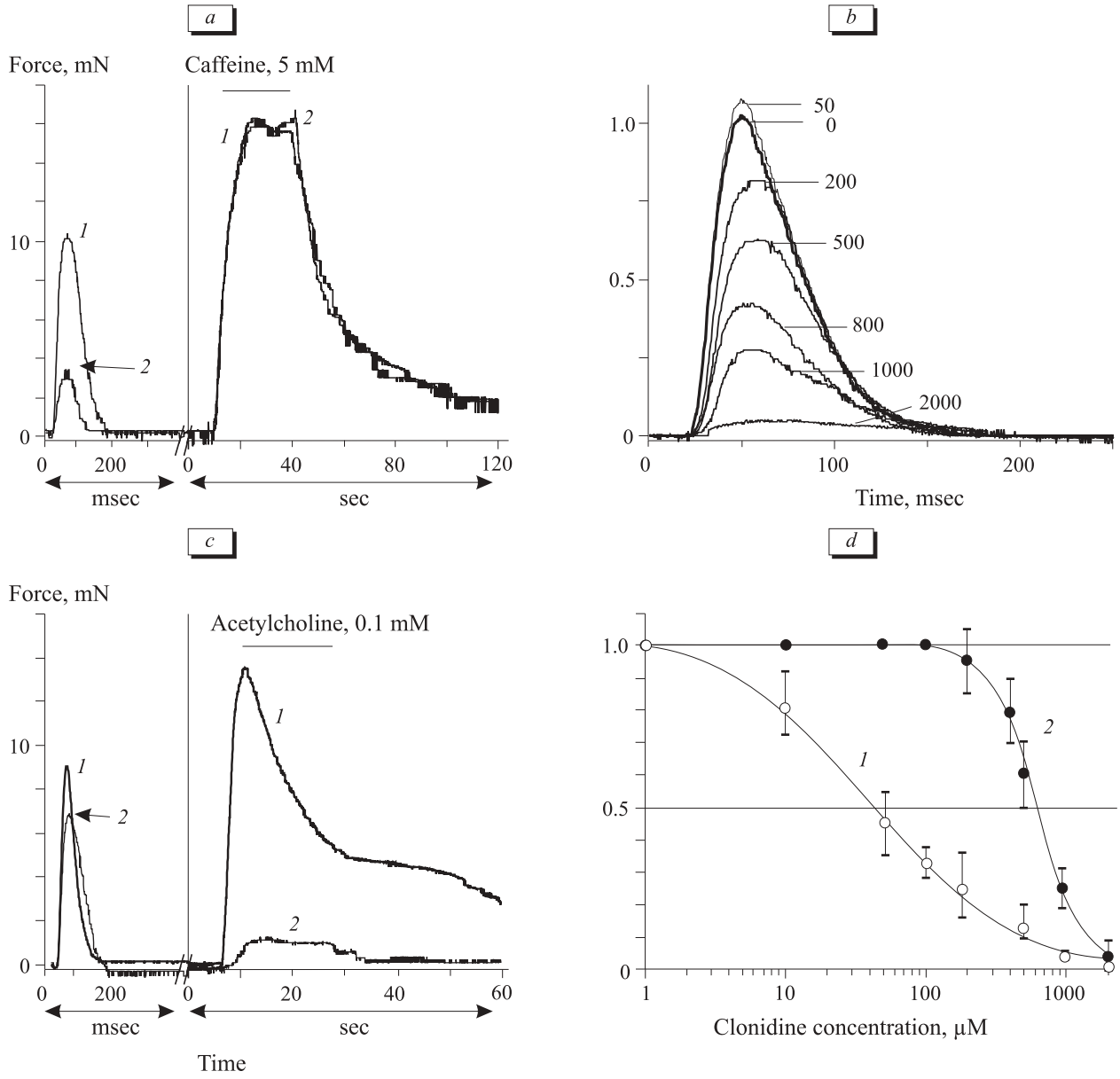

Fig. 2. Effect of clonidine on contractile responses of frog skeletal muscle. *a*) single contraction (SC) and caffeine contraction before (*1*) and after exposure to clonidine (1000 μM) for 20 min (*2*); *b*) SC during direct electrical stimulation in clonidine-free (0 μM) control solution and after successive application of clonidine in concentrations of 50, 200, 500, 800, 1000, and 2000 μM; the exposure time was 20 min for each concentration; *c*) SC and acetylcholine contraction before (*1*) and after 20-min exposure to 400 μM clonidine (*2*); *d*) the dependence of the inhibitory effect of clonidine on the amplitude of SC (*1*) and acetylcholine contraction (*2*). Ordinates: *b*, *d* — the maximum force of contraction normalized to control response. The solid lines were calculated according to Hill equation with the following parameters: *1*) IC_{50}=645 μM, nH=2.44 and *2*) IC_{50}=44 μM, nH=1.

extremely high density of Na^+ channels [6]. Therefore, the action potential in these fibers can be generated even when 50% channels are blocked. It could be hypothesized that the blocking action of clonidine on Na^+ channels in skeletal muscles is realized in the same concentration range as in TTX-resistant Na^+ channels of sensory neurons with IC_{50} about 200-300 μM.

The contractile responses induced by acetylcholine were most sensitive to the blocking action of clonidine (Fig. 2, *c*). A pronounced drop in acetylcholine-induced contraction was observed at concentrations of 25-50 μM. The dose-dependence of the inhibitory effect of clonidine on acetylcholine-induced contraction is described by Hill equation with IC_{50}=44 μM and nH=1 (Fig. 2, *d*). Since the data on the effect of clonidine on caffeine and potassium contractions showed that this agent does not affect the excitation-contraction coupling system and the maximum force developed by frog muscle during acetylcholine-induced contraction practically does not depend on functional

activity of Na^+ channels [2], a direct blocking action of clonidine on acetylcholine-controlled channels of plasmalemma in 1:1 stoichiometry could be suggested on the basis of Hill coefficient nH=1.

Thus, we revealed similarity between LA and clonidine molecules by the spatial and electron structures. The linear sizes of clonidine molecule allow its incorporation into Na^+ channel pore at the binding site of LA (Y1771) due to π-electron interaction of the aromatic ring in contrast to LA molecule, which incorporates into the pore due to π-electron binding with Y1771 site and hydrophobic interaction with F1764 site.

The revealed peculiarities were corroborated by experimental study of the effects of clonidine on contractile responses of skeletal muscle: the absence of direct effect on Ca^{2+} release from the sarcoplasmic reticulum, poor effect on SC depending on functional activity of Na^+ channels, and the greatest efficiency of clonidine towards contraction response induced by acetylcholine.

It can be hypothesized that combination of the blocking effect of clonidine on cholinoreceptors (IC_{50}= 40 μM) and its inhibiting effect on TTX-resistant Na^+ channels (IC_{50}=100 μM), pronounced modulation of the conduction of nociceptive signal underlying analgesic effect would be attained at far lower concentrations of this drug (0.01-0.10 μM), which are comparable to those used in clinical practice.

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