Isolated viagra solution application effects on the electrical activity in Helix neurons

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Abstract: The authors explored effects of applying an isolated solution of Viagra (Sildenafil Citrate) on the electrical activity in unidentified neurons of the Helix visceral ganglion. At a concentration of $0.5 \times 10^{-2}$ M, the neurotropic effect of Viagra was present in the form of a persistent hyperpolarization of the neuron membrane, resulting in reduced neuronal impulses, with the membrane potential not reaching its baseline values upon washing out. At a concentration of $10^{-3}$ M, the effects were also inhibitory, accompanied by a non-significant cell membrane depolarization. At a concentration of $10^{-5}$ to $10^{-4}$ M, Viagra produced excitatory effects, with the membrane potential reaching its baseline values upon washing out. The discovered data on the influence and neurotoxicity of Viagra are to be accounted for when administering its specific dosages, to prevent possible negative effects. Further research is needed to expand the knowledge on possible clinical effects of Viagra administering.

1. Introduction

There is a long history of research on the clinical use of Viagra [1]. From the very start, researchers studied antianginal properties of the drug, however no significant effects were found. Further on, it was proven to enhance erectile function via inhibition of phosphodiesterase type 5 [2]. Some authors believe [3–5] that Viagra possesses a capacity to modulate interneuronal communication. However, in the literature, we could not find specific information on Viagra’s capacity to modulate functioning of the nervous system in general, and its structural and functional basis – neurons in particular. No relevant data is available on the direction and mechanisms of neurotropic effects of this drug in different dosages. It is to be noted though, that our earlier research already demonstrated the excitatory neurotropic effects of Viagra at a concentration of $10^{-4}$ M [6].

Hence, given the wide range of clinical applications for Viagra (in pulmonology, cardiology, gastroenterology, gynecology, etc.) [7] and the lack of specific knowledge on its neurotropic effects, we aimed our research at studying Viagra effects (their direction, mechanism, optimal and toxic dosage thresholds) when the substance is applied to the membrane of neurons at different concentrations.

2. Methods

Viagra’s structural formula is given in Figure 1. We analyzed the effects of its application to unidentified neurons at concentrations of $0.5 \times 10^{-2}$ M (n = 15), $10^{-3}$ M (n = 20), $10^{-4}$ M (n = 20), and...
$10^5$ M (n = 30). The Shilpa Medicare Limited (India) sample of sildenafil citrate (lot NPV0080309, content per base) was used.

![Structural formula of Viagra (sildenafil citrate).](image)

Figure 1. Structural formula of Viagra (sildenafil citrate).

The experimental procedure was based on the use of the commonly accepted technique for recording intracellular biopotentials [8]. The recording and analysis of each neuron’s activity were conducted using a special computer software [9] according to the following protocol: baseline, 1-1.5 minutes; exposure to a specific concentration of the substance – 5-6 minutes, and 20 minutes – washing out. The biopotential measurements were averaged over each recording period. The following electrical activity indices in the studied neurons were analyzed: pulse generation frequency (PGF), action potential (AP) duration and amplitude, increase rate maxima for the total of inward and outward ion currents, and resting membrane potential (MP) value.

The collected data underwent statistical processing with the nonparametric Wilcoxon test. The data are presented in relative values (%), means ± mean error.

3. Results

The application of Viagra at a concentration of $0.5 \times 10^{-2}$ M blocked generation of both full-amplitude and reduced APs in all examined neurons (Figure 2, A). The MP sharply shifted toward hyperpolarization by 10-12 mV and remained at a new level both during exposure (see Figure 2, A) and upon washing out stages. The Figure 2, A demonstrates that both inward and outward ion currents are nearly fully blocked. To sum up, in such a concentration, Viagra renders inhibitory effects and irreversibly blocks the AP generation mechanism, while the mechanism of MP retention is still preserved. Upon washing out, the impulse activity in neurons does not recover.

For a condition of applying Viagra at a concentration of $10^{-3}$ M, a typical neurogram is given in Figure 2, B, demonstrating a pronounced inhibition of neuron functional state indicative of which were an increase in the neuron MP, growing hyperpolarization and a decrease in the AP (on average, the sample value was lower by 16 % relative to the baseline (see Figure 3, column 5)). The analysis of the first derivative revealed that the total inward and outward currents (see Figure 2 and 3), compared to the baseline, decreased to $78.7 \pm 10.1\%$ and $73.6 \pm 9.7\%$, p < 0.05, respectively, which naturally led to the AP inhibition (Figure 2 B and Figure 3, columns 3 and 4).

When applied at a concentration of $10^{-4}$ M, Viagra produced a light hyperpolarization of the membrane and a decrease in the AP amplitude (by 5-10mV) and PGF, 1.75 times (Figure 2, C). At 50-70 sec of the agent exposure, the PGF rapidly increased and, at 100-150 sec, exceeded the baseline value by 150-200% (p < 0.05). The analysis of the first derivative showed (Figure 2, C) that, at such a concentration, Viagra considerably accelerates the rate of increase in total inward and decrease in total outward ion currents (Figure 3, columns 3 and 4, respectively). At the same time, most neurons demonstrated a strong membrane depolarization (by 11.2% on average) (Figure 3, column 6).
Neurotropic effects of Viagra at a concentration of $10^{-5}$ M were as follows. At 20-30 sec after the application occurred, the MP slightly (by 2-5 mV) shifts to depolarization accompanied by a sharp 1.5-2 times increase in the PGF and by $12 \pm 5\%$ ($p \leq 0.05$) in the AP amplitude (Figure 3, column 5). The analysis of the AP first derivative revealed that Viagra, applied at the given concentration, evokes a significant ($p < 0.05$) growth in the maximum rate of increase of the total inward ion currents, and in the rate of the AP decrease (Figure 2, D, Figure 3, columns 3, 4). In other words, Viagra, applied at such a concentration, facilitates the permeability of membrane for Na⁺, and then for the outward ion currents.

**Figure 2.** Examples of neurotropic effects produced by Viagra exposure at different concentration levels (A – D) on the electrical potentials in the visceral ganglion unidentified neurons; A′, B′, C′, D′ – 1 – mean AP, 2 – its first derivative. The neurogram averaged fragments are underlined with a horizontal line; the arrow marks the application starting point.
4. Discussion

The analysis of the functional state in neurons exposed to Viagra at different concentration levels revealed its pronounced neurotropic effects manifested in certain changes observed for all electrophysiologic indicators in neurons.

Viagra application effects strongly depended on its concentration levels: the higher the concentration level, the more pronounced the decrease in the AP amplitude: at $0.5 \times 10^{-2}$ M concentration level, Viagra fully blocks ionic currents responsible for generating both the background APs, and those evoked by a single intracellular stimulation with a depolarizing current. At $10^{-3}$ concentration level, Viagra application produces a less pronounced yet statistically significant fall in the rate of increase of the inward and outward ion currents.

At $10^{-4}$ to $10^{-5}$ concentration levels, Viagra produces excitatory effects manifested in a considerable PGF increase. The analysis of the AP first derivative demonstrated that, at the given concentration levels, Viagra facilitates membrane permeability for the inward ion currents. Since Viagra manifests itself in inhibiting phosphodiesterase type 5 [10], present in the postsynaptic membranes of nervous tissue at an extremely high level, it can be assumed that the mechanism underlying its excitatory effects can be based on the inhibition of the phosphodiesterase enzyme activity, which apparently causes an increase in cAMP content, and higher rates of metabolism in neurons. As a result, there is an increase in membrane permeability in neurons for Na$^+$ ions, in that accelerating the phase of depolarization, reducing its critical level and significantly increasing the neuron pulse activity.

As Viagra blocks phosphodiesterase type 5, one might assume that a gradual increase in its concentration would regularly produce stronger excitatory effects. However, such dependency occurs only for relatively low concentration levels. In our opinion, at higher concentration levels, the observed effects are based on a different mechanism, with Viagra affecting not only the metabolism in the cell, as it is the case for $10^{-5}$ to $10^{-4}$ M concentration levels, but rather the fast processes in the cell,
i.e., binds to receptors and directly affects ion channels. We believe that, at higher concentration levels, Viagra effects can be accounted for its capacity to establish strong connections with membrane receptors. Since the observed effects are inhibitory, Viagra seems to evoke inhibitory processes [11] resulting in blocked electrical activity in neurons, with their membrane ionic conductivity to be irreversibly damaged.

5. Conclusions
To sum up, Viagra application can evoke both excitatory and inhibitory effects on neurons, depending on its concentration levels. At higher concentration levels, such as $10^{-3}$ M and $0.5 \times 10^{-2}$ M in particular, Viagra produces pronounced neurotoxic effects. The obtained data on Viagra’s effects and its neurotoxicity must be taken into consideration when administering its certain dosages, to prevent possible negative side effects. Further research is needed to collect more detailed knowledge on Viagra application effects and consequences of its administering as a drug with a wide clinical use.

6. References

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