Artículo de Investigación/Research Article

CO, Pb++ and SO2 effects on L-type calcium channel and action potential in human atrial myocytes. In silico study

Efectos del CO, Pb++, y SO2 en el canal de calcio tipo L y potencial de acción en miocitos auriculares humanos. Estudio en silico

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Abstract

Exposure to air pollutants like carbon monoxide (CO), lead (Pb^{++}) and sulfur dioxide (SO_2) promotes the occurrence of cardiovascular diseases. Experimental studies have shown that CO, Pb^{++} and SO_2 block L-type calcium channels, reducing the calcium current (I_{CaL}) and the action potential duration (APD), which favors the initiation of atrial arrhythmias. The goal is to study the effects of CO, Pb^{++} and SO_2 at different concentrations on I_{CaL} and action potential using computational simulation. For this purpose, models of the effects of the air pollutants on the atrial L-type calcium channel were developed and were incorporated into a mathematical model of a human atrial cell. The results suggest that CO, Pb^{++} and SO_2 block the I_{CaL} current in a fraction that increases along with the concentration, generating an APD shortening. These results are consistent with experimental studies. The combined effect of the three air pollutants produced an APD shortening, which is considered to be a pro-arrhythmic effect.

Keywords

Air pollution, atrial action potential, calcium channel, in silico models.

Resumen

La exposición a contaminantes atmosféricos, como el monóxido de carbono (CO), plomo (Pb^{++}) y dióxido de azufre (SO_2), promueve la aparición de enfermedades cardiovasculares. Estudios experimentales han demostrado que el CO, el Pb^{++} y el SO2 bloquean los canales de calcio tipo L, disminuyendo la corriente de calcio (I_{CaL}) y la duración del potencial de acción (APD), favoreciendo el inicio de arritmias auriculares. El objetivo es estudiar los efectos del CO, Pb^{++} y SO_2 a diferentes concentraciones, sobre I_{CaL} y el potencial de acción auricular mediante simulación computacional. Para ello, se desarrollaron modelos matemáticos de los efectos de los contaminantes atmosféricos sobre el canal de calcio auricular tipo L y se incorporaron en un modelo matemático de células auriculares humanas. Los resultados sugieren que el CO, el Pb^{++} y el SO_2, bloquean la corriente I_{CaL} en una fracción que aumenta a medida que aumenta, la concentración, generando un acortamiento del APD. Estos resultados son consistentes con estudios experimentales. El efecto combinado de los tres contaminantes atmosféricos generó un acortamiento del APD, lo cual es considerado un efecto pro-arrítmico.

Palabras clave

Contaminantes atmosféricos, potencial de acción auricular, canal de calcio, modelos en silico.

1. INTRODUCTION

Air pollution is defined as the presence in the atmosphere of one or more substances in sufficient quantity to produce health alterations. Air pollution causes 4.3 million premature deaths annually [1]. In 2010, the economic cost of the impact of air pollution on health in developing countries was around USD 1.7 billion [1].

Carbon monoxide (CO) is a toxic gas that results from incomplete combustion. When people breath, the CO binds to hemoglobin and it is retained in the blood, significantly reducing the amount of oxygen that it can transport [2]. Lead (Pb^{++}) is a toxic agent that can exert adverse effects on human health. According to the United States Environment Protection Agency (EPA) Pb^{++} is one of the most dangerous air pollutants, affecting multiple human body systems [3]. In general, more than 143.000 people die every year due to illnesses related to Pb^{++} [4]. Sulfur dioxide (SO₂) is an invisible gas that has a nasty, sharp smell. The main source of SO₂ in the air is industrial activity that involves processing materials that contain sulfur. It causes cardiovascular diseases and cardiovascular mortality [5].

Exposure to these air pollutants contributes to cardiovascular diseases [6]. Epidemiological studies have reported effects such as heart failure, generation of cardiac arrhythmias and decreased heart rate variability [3-13]. Experimental studies have shown that Pb^{++} blocks L-type calcium channels [14]. A decrease in L-type calcium current (I$_{CaL}$) is an important mechanism that favors the generation of atrial arrhythmias [15]. Recently, it has been shown that chronic exposure to CO promotes a pathological phenotype of cardiomyocytes in which remodeling and intracellular Ca^{++} overload increase the risk of arrhythmias [10, 12] and ischemia [16]. On the other hand, experimental studies suggest that exposure to SO₂ produces an effect on the I$_{CaL}$ current that can produce damage in cardiomyocytes, which in turn results in hypoxia and ischemia [17, 18]. The objective of this work is to study, through computer simulation, the electrophysiological effects of Pb^{++}, CO and SO₂ at different concentrations on I$_{CaL}$ and action potential handset under normal electrophysiological conditions.

2. METHODOLOGY

2.1 Human atrial cell model

The Courtemanche Ramirez Nattel Kneller [19, 20] membrane formalism was implemented to simulate the electrical activity of human atrial cell. The transmembrane voltage (V$_m$) is given by (1):

$$C_m \frac{dV_m}{dt} + I_{ion} + I_{st} = 0 \qquad (1)$$

Where C$_m$ is the membrane capacitance (100 pF), I$_{ion}$ is the total membrane current and I$_{st}$ is the external stimulus current. The model is considered under normal electrophysiological conditions.

2.2 Model of CO effect on I$_{CaL}$

Based on an experimental study [16], a relationship between CO concentration and APD decrease was established. This study attributes the APD decrease to a blockage of the I$_{CaL}$ current; therefore, a basic model of CO's effect on the I$_{CaL}$ current was developed. The blocking factor (bco) of the I$_{CaL}$ current by CO depends on the concentration of CO (D) and has a lineal behavior. This equation was introduced in the I$_{CaL}$ equation of the cell model, and it was adjusted to obtain APD reductions approximately equal to those observed experimentally.

The equations to calculate bco and I$_{CaL}$ are given by Eq. (2) and (3):

$$b_{CO} = 0.0002D + 0.0827 \qquad (2)$$

$$I_{CaL} = (1 - b_{CO})g_{CaL}dff_{Ca}(V_m - 65) \qquad (3)$$

Where g$_{CaL}$ is the maximum conductance of I$_{CaL}$, d is the activation gate, f is the voltage-dependent inactivation gate, f$_{Ca}$ is the calcium-dependent inactivation gate and 65 is the value of equilibrium potential for I$_{CaL}$.

2.3 Model of Pb^{++} effect on I$_{CaL}$

To develop a basic model of the Pb^{++} effect on I$_{CaL}$, the steady-state fraction of block (b$_{Pb}$) was used. Hill's equation has been used to fit the concentration-response relationships for ligand block. It describes the fraction of the macromolecule saturated by ligand as a function of the ligand concentration; it is used for determining the degree of cooperativeness of the ligand binding to the receptor. In this model, the kinetics of the channel would be considered unchanged in the presence of the Pb^{++}, Eq. (4).

$$b_{Pb} = \frac{1}{\left[1 + \frac{IC_{50}}{D}\right]^h} \qquad (4)$$

Where IC$_{50}$ is the half maximal inhibitory concentration for the current block by Pb^{++} and D is the Pb^{++} concentration. A Hill coefficient (h) of 1 indicates completely independent binding. A total of 152 nM was used for the IC$_{50}$ to block I$_{CaL}$; this value was found by [14] in ventricular

myocytes. There are no reported values of IC$_{50}$ for blocking channel by Pb^{++} on atrial myocytes.

The following is the modified equation to simulate the I$_{CaL}$ current

$$I_{CaL} = (1 - b_{CO})(1 - b_{Pb})g_{CaL}dff_{Ca}(V_m - 65) \quad (5)$$

2.4 Model of SO$_2$ effect on I$_{CaL}$

Based on experimental studies [17] [18], a basic model of the SO$_2$ effect on I$_{CaL}$, current was developed. The decrease factor of I$_{CaL}$, by SO$_2$ (b$_{SO2}$) depends on the SO$_2$ concentration (D) [17], as follows, Eq. (6):

$$b_{SO_2} = \frac{1}{\left[1 + \frac{35.99}{D}\right]^{1.0}} \qquad (6)$$

The modified equation to simulate the I$_{CaL}$ current is (7).

2.5 Simulation protocol and data analysis

Unicellular models to simulate the sinus rhythm under physiological conditions were implemented using the Cellular Open Resource public CellML OpenCOR® software. Forward Euler's method, with a time step of 0.001 ms, was implemented to solve the equations. A train of 10 stimuli was applied. The basic cycle length was 1000 ms. Concentrations of CO from 0 to 1000 µM, of Pb^{++} from 0 to 300 nM and of SO$_2$ from 0 to 200 µM were implemented.

APD at 90% of the repolarization (APD$_{90}$) as well as I$_{CaL}$ and I$_{Na}$ currents were measured. All measurements were made on the 10th beat using a program developed in MATLAB® software

$$I_{CaL} = (1 - b_{CO})(1 - b_{Pb})(1 - b_{SO_2})g_{CaL}dff_{Ca}(V_m - 65) \qquad (7)$$

3. RESULTS AND DISCUSSION

3.1 CO effects on I$_{CaL}$ and action potential

Fig. 1 shows the effects of different CO concentrations on the Fig. 1 shows the effects of different CO concentrations on the I$_{CaL}$ current and atrial action potential under normal electrophysiological conditions. It is possible to observe that I$_{CaL}$ without CO shows a peak of -449 pA and the current remains active during the plateau phase of action potential. As the CO concentration increases, I$_{CaL}$ downregulation is observed, which causes an APD shortening (Table 1) and loss of plateau phase. When the highest CO concentration was applied (1000 μM), the I$_{CaL}$ peak de-

creased 21% (-356 pA) and the APD$_{90}$ reached a value of 151 ms, which indicates a decrease of 28%.

Table 1 shows the APD$_{90}$ decreasing values as a function of the different CO concentrations. The increase in CO concentration generated an APD shortening.

Table 1. APD$_{90}$ at different CO concentrations.
Source: Authors.

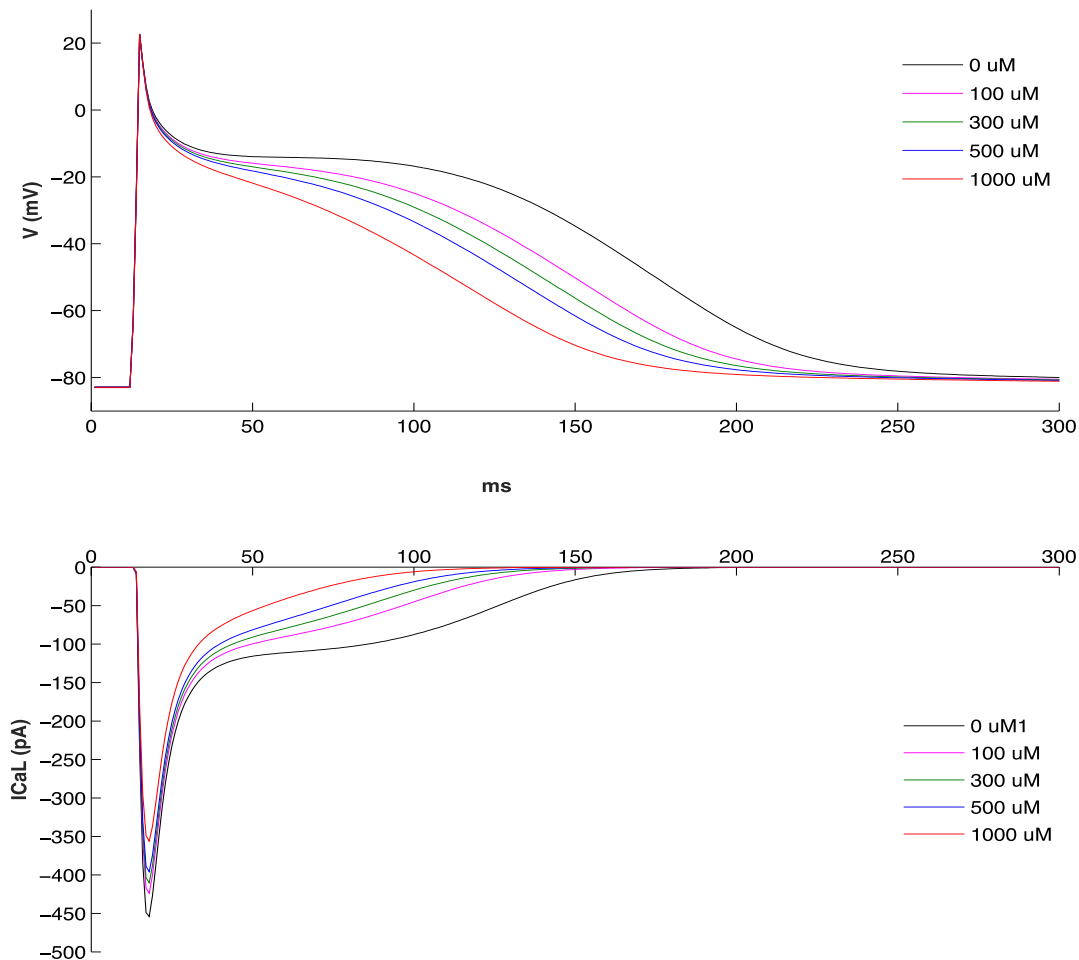| [CO]	APD$_{90}$
0 μM	211 ms
100 μM	187 ms
300 μM	177 ms
500 μM	169 ms
1000 μM	151 ms

Fig. 1. Effects of CO at different concentrations (from 0 to 1000 μM) on action potential (top) and I$_{CaL}$ current (bottom).
Source: Authors.

3.2 Pb^{++} effects on I$_{CaL}$ and action potential

Fig. 2 shows the effects of different Pb^{++} concentrations on I$_{CaL}$ current and atrial action potential under normal electrophysiological conditions. It can be observed that I$_{CaL}$ without Pb^{++} shows a peak of -454 pA and the current remains active during the plateau phase of action potential. As with CO, while Pb^{++} concentration increases, I$_{CaL}$ downregulation is observed, which causes an APD shortening (Table 2) and loss of plateau phase. When the highest Pb^{++} concentration was applied (300 nM), the I$_{CaL}$ peak decreased 58% (-191 pA) and the APD$_{90}$ reached a value of 115 ms, which indicates a decrease of 45%.

Table 2 shows the APD$_{90}$ decreasing values as a function of the different Pb^{++} concentrations. The increase in Pb^{++} concentration generated an APD shortening.

Table 2. APD$_{90}$ at different Pb^{++} concentrations. Source: Authors.

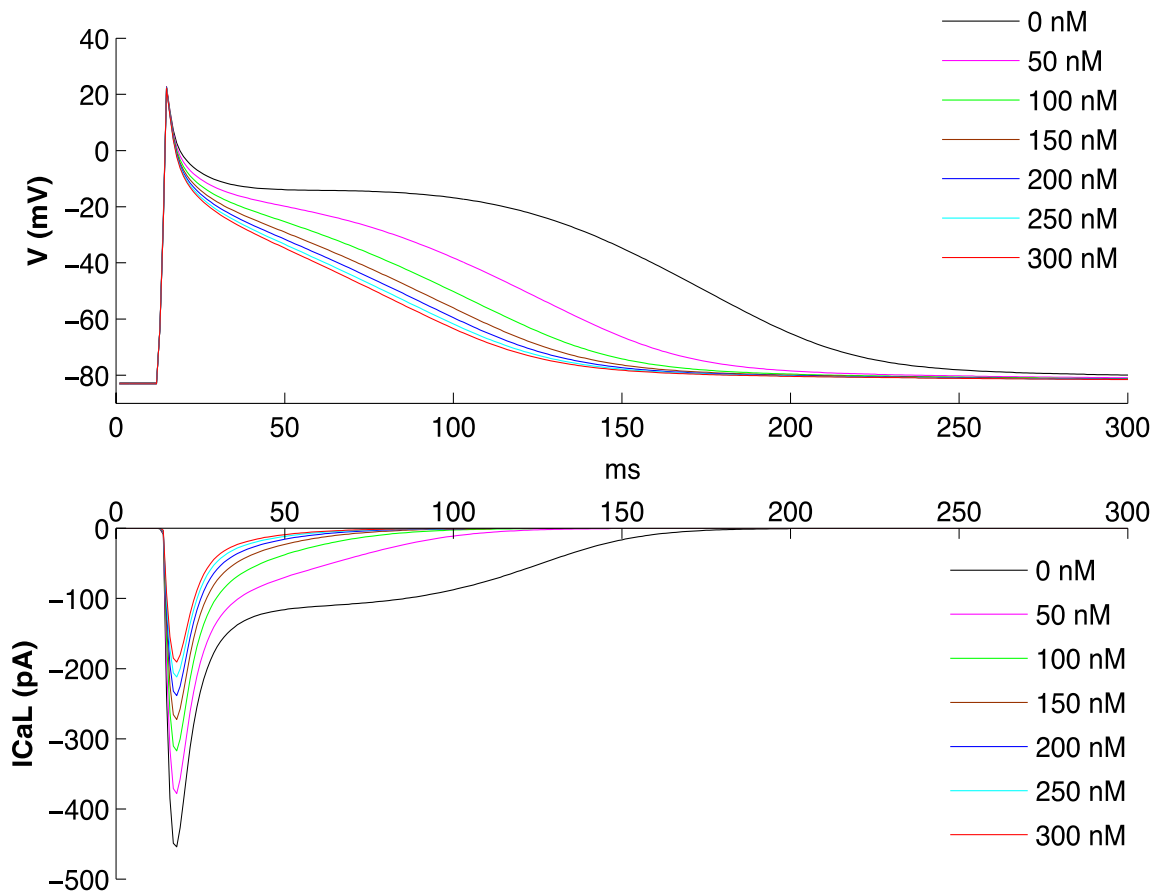| [Pb++]	APD$_{90}$
0 nM	211 ms
50 nM	160 ms
100 nM	139 ms
150 nM	129 ms
200 nM	122 ms
250 nM	118 ms
300 nM	115 ms

Fig. 2. Effects of Pb^{++} at different concentrations (from 0 to 300 nM) on action potential (top) and I$_{CaL}$ current (bottom). Source: Authors.

3.3 SO$_2$ effects on I$_{CaL}$ and action potential

Fig. 3 shows the effects of different SO$_2$ concentrations on I$_{CaL}$, I$_{Na}$ and atrial action potential under normal electrophysiological conditions. It is possible to observe that I$_{CaL}$ without SO$_2$ shows a peak of -454 pA and this current remains active during the plateau phase of action potential. When the highest concentration of SO$_2$ was applied (1000 µM), the I$_{CaL}$ peak creased 96% (-19.2 pA) and the APD$_{90}$ reached a value of 80 ms, which indicates a decrease of 62% (Table 3).

3.4 SO$_2$ effects on I$_{CaL}$ and action potential

The results of this section show the combined effects of the three air pollutants

Table 3. APD90 at different SO2 concentrations. Source: Authors.

[SO2]	APD90
0 µM	211 ms
5 µM	183 ms
50 µM	120 ms
100 µM	107 ms
500 µM	82 ms
1000 µM	80 ms

CO, Pb^{++} and SO$_2$, at different concentrations on the I$_{CaL}$ and action potential, under normal electrophysiological conditions. It can be observed in Fig. 4, that I$_{CaL}$ without pollutants shows a peak of -454 pA, this current remains active during the plateau phase of action potential. When the highest concentrations of CO (1000
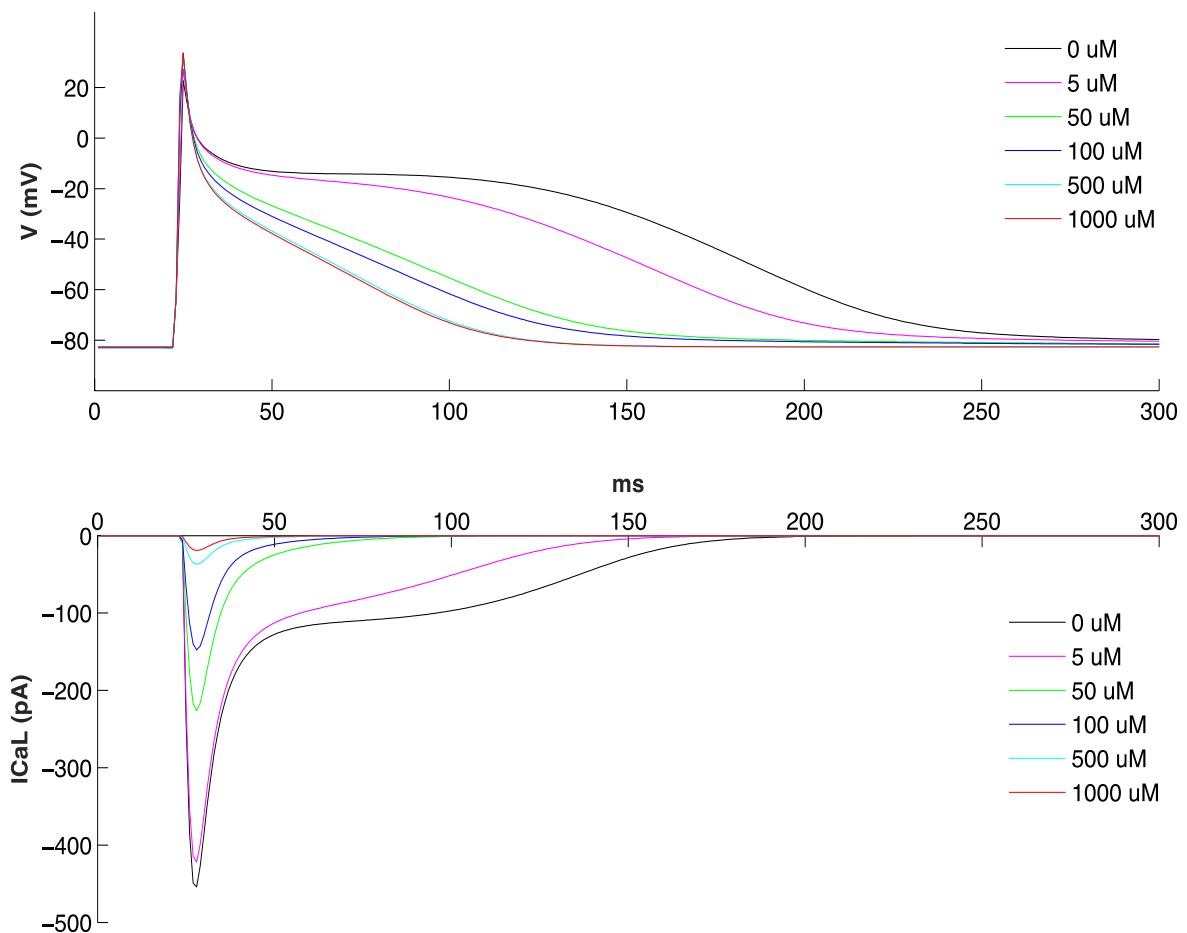

Fig. 3. Effects of SO$_2$ at different concentrations (from 0 to 1000 µM) on action potential (top) and I$_{CaL}$ current (bottom). Source: Authors.

μM), Pb^{++} (300 nM) and SO$_2$ (1000 μM) where applied, the I$_{CaL}$ peak decreased 99% (-4.9 pA) and the APD$_{90}$ reached a value of 79 ms, which indicates a decrease of 63% (Table 4). The effects of the three air pollutants in combination caused an important APD decrease.

Table 4 shows the APD$_{90}$ decreasing values in function of the different concentration of the three air pollutants. CO, Pb^{++} and SO$_2$ concentrations increases generate an APD shortening.

The results showed that CO blocks the I$_{CaL}$ current in a greater fraction as CO concentration increases. These results are consistent with experimental studies. A study in rats [16] showed that CO leads to an important reduction of the APD in atrial myocardium, as well as a significant acceleration of the sinus rhythm and reduction in the force of contraction. Another study in rats showed that CO inhibits native rat cardiomyocyte L-type Ca^{2+} currents

[21]. Additionally, it has been demonstrated that CO pollution promotes cardiac remodeling and ventricular arrhythmia in healthy rats [22]. A recent study using mathematical simulations showed that CO causes early post-depolarizations in the ventricular action potential [23] due to effects on the sodium and calcium currents, which demonstrates its proarrhythmic effect. Our results are consistent with these studies: as soon as the concentration of CO increases, the APD shortening is observed under normal physiological conditions. There are no in silico studies of the effects of CO on human atrial action potential.

The results also showed that Pb^{++} blocks the I$_{CaL}$ current in a greater fraction as the concentration increases. This extends its action in time, which results in an APD shortening, as experimentally demonstrated [14]. This analysis is consistent
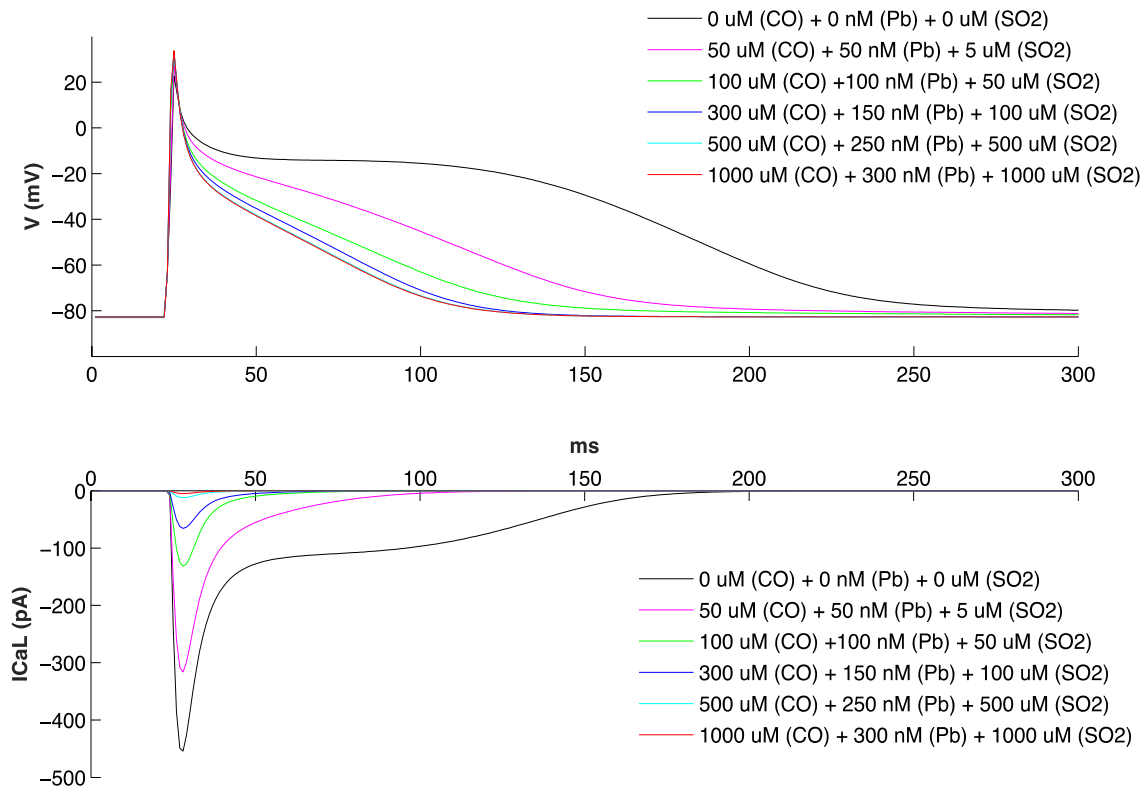

Fig. 4. Effects of the CO, Pb^{++} and SO$_2$ at different concentration on action potential (top) and ICaL current (botton).
Source: Authors.

CO, Pb^{++}, and SO$_2$ effects on l-type calcium channel and action potential in human atrial myocytes.
In silico study

Table 4. APD90 at different CO, Pb++ and SO$_2$ concentrations. Source: Authors.

[CO]	[Pb++]	[SO2]	APD90
0 μM	0 nM	0 μM	211 ms
50 μM	50 nM	5 μM	138 ms
100 μM	100 nM	50 μM	104 ms
300 μM	150 nM	100 μM	84 ms
500 μM	250 nM	500 μM	80 ms
1000 μM	300 nM	1000 μM	79 ms

with an experimental study of ventricle myocytes of rats [14]. In it, Pb^{++} blocked the L-type calcium channels; however, its mechanism is not well explained. A study of the ventricular myocardium of rats suggests that acute lead administration reduces the myocardial contractility by reducing sarcolemmal calcium influx and the myosin ATPase activity [24]. It has been observed that Pb^{++} may alter the bioelectrical properties of neurons by blocking high-voltage activated Ca^{2+} currents, particularly L-type [25], and differentially blocking the cloned T-type calcium channels [26]. There are no in silico studies of the effects of Pb^{++} on human atrial action potential.

Similarly, the results showed that SO$_2$ also blocks the I$_{CaL}$ current in a greater fraction as the concentration increases, which results in ADP reductions. Experimental studies of ventricular myocytes of rats [17, 18] showed that exposure to SO$_2$ causes a decrease of the I$_{CaL}$ current and an increase of the I$_{Na}$ current, which can produce damage in cardiomyocytes and result in hypoxia and ischemia. Our results are consistent with these studies: when the concentration of SO$_2$ increases, the APD decreases under normal physiological conditions. There are no in silico studies of the effects of SO$_2$ on human atrial action potential.

When the combined effects of the three air pollutants, CO, Pb^{++} and SO$_2$, at different concentrations on I$_{CaL}$ were simulated, the results showed a significant APD decrease in a greater fraction as the concentrations increase, which is a severe pro-

arrhythmic effect. Clinical studies have shown that air pollution increases the risk of cardiovascular disease mortality by 76 % [27]; such deaths are mainly related to ischemia, arrhythmias and heart failure [23–25]. Sufficient evidence has been found to conclude that a brief exposure to high levels of pollutants increases the patients' cardiac mortality. Likewise, it has been shown that prolonged exposures reduce people's life expectancy by several years and hospital admissions due to cardiovascular diseases increase with high concentrations of pollutants [31]. Recent studies have been able to demonstrate that cardiac arrhythmias present higher probability of occurrence after the exposure to air pollutants, thus concluding that air pollution is an acute "trigger" of these arrhythmias [32]. Despite the existence of studies of the effects of air pollutants on the cardiovascular system in the literature, the mechanisms underlying the effects of acute and chronic exposure to these agents on the heart have not been well established. In silico studies may contribute to a better understanding of the mechanisms by which air pollutants have unhealthy effects on cardiac tissue, promoting cardiac diseases such as arrhythmias.

4. CONCLUSIONS

Our results are in agreement with experimental studies that have demonstrated that CO, Pb^{++} and SO$_2$ block the I$_{CaL}$ current in a greater fraction as the concentration increases, which results in an APD

shortening. It was possible to simulate the combined effects of the three air pollutants with models. The results showed an important APD decrease that suggests a relevant pro-arrhythmic effect.

This in silico study contributes with research into the electrophysiological effects of CO, Pb^{++} and SO$_2$ on the atrial action potential of healthy people. To our knowledge, this is the first work that has developed mathematical models that evaluate the effect of air pollutants on human atrial currents and action potential.

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