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# The electrophysiological effect of cannabidiol on hERG current and in guinea-pig and rabbit cardiac preparations

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Cannabis use is associated with cardiovascular adverse effects ranging from arrhythmias to sudden cardiac death. The exact mechanism of action behind these activities is unknown. The aim of our work was to study the effect of cannabidiol (CBD), tetrahydrocannabinol and 11-nor-9-carboxy-tetrahydrocannabinol on cellular cardiac electrophysiological properties including ECG parameters, action potentials, hERG and  $I_{Kr}$  ion channels in HEK cell line and in rabbit and guinea pig cardiac preparations. CBD increased action potential duration in rabbit and guinea pig right ventricular papillary muscle at lower concentrations (1  $\mu\text{M}$ , 2.5  $\mu\text{M}$  and 5  $\mu\text{M}$ ) but did not significantly change it at 10  $\mu\text{M}$ . CBD at high concentration (10  $\mu\text{M}$ ) decreased inward late sodium and L-type calcium currents as well. CBD inhibited hERG potassium channels with an  $\text{IC}_{50}$  value of 2.07  $\mu\text{M}$  at room temperature and delayed rectifier potassium current with 6.5  $\mu\text{M}$  at 37 °C, respectively. The frequency corrected QT interval ( $\text{QT}_c$ ) was significantly lengthened in anaesthetized guinea pig without significantly changing other ECG parameters. Although the  $\text{IC}_{50}$  value of CBD was higher than literary  $\text{C}_{\text{max}}$  values after CBD smoking and oral intake, our results raise the possibility that hERG and potassium channel inhibition might have a role in the possible proarrhythmic adverse effects of cannabinoids in situations where metabolism of CBD impaired and/or the repolarization reserve is weakened.

Cannabis is the most abused hallucinogenic drug, with an estimated of 150 million consumers worldwide<sup>1</sup>. With the increasingly widespread use of e-cigarettes, the number of people inhaling cannabinoids might even be higher. Moreover, the use of cannabis products for medicinal purposes is increasing globally. The interest for the use of cannabis and cannabis-derived products started following the discovery of the cannabinoid system in the human brain and body and the subsequent reports on new findings on biological activities of cannabinoids on central nervous system and immune functioning. Currently, there are cannabis-based medicines on the market with well-defined medicinal indications, including treatment of nausea and vomiting associated with chemotherapy, anorexia, pain related to cancer, spasticity and pain associated with multiple sclerosis, Dravet and Lennox-Gastaut syndromes. These medicines contain known amounts of CBD and/or THC in pure form or as standardized herbal extract<sup>2</sup>. Besides, the use of CBD-containing products (CBD oil) is very widespread with several, clinically unsupported indications. The intake of cannabinoids, especially CBD, which is enriched in several products, may be higher in case of the consumption of CBD oils than in case of smoking cannabis.

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The cardiovascular adverse effects of cannabinoid use have been reported in several case reports, and range from arrhythmias to myocardial infarction and sudden death<sup>3</sup>. According to the results of a cohort study, marijuana smokers have a 4.8-fold increased risk of developing acute myocardial infarction during the first hour of exposure<sup>4</sup>. However, other data do not support the association between cannabis use and cardiovascular events<sup>5</sup>. The most comprehensive study assessed data for 316,397 cannabis users and 20,499,215 non-users, and found that cannabis use is an independent predictor of heart failure<sup>1</sup>. Although the exact mechanisms explaining these observations are unknown, the activities of cannabinoids exerted via the G protein-coupled cannabinoid receptors are supposed to be of key importance. In addition, several studies described the proarrhythmic potency of cannabinoids ranging from ventricular arrhythmias to sudden cardiac death<sup>6–8</sup>. However, the exact association and mechanism of these arrhythmias remain unknown<sup>7</sup>. Besides, certain voltage-gated ion channels like cardiac sodium, calcium<sup>9</sup> and Kv4.3 channels<sup>10</sup> might also be related to the reported cardiovascular effects of cannabinoids, but the exact role of these channels has not been studied yet in detail. One of the most important ion channels in cardiac repolarization is the rapid delayed rectifier potassium channel ( $I_{Kr}$ ), which plays a critical role in cardiac repolarization, having a pore-forming subunit encoded by the *hERG* (the human Ether-à-go-go-Related Gene) gene. Inhibitors of  $I_{Kr}$  (also called hERG ion channel) are known to lengthen the QT interval, and hence might induce life-threatening arrhythmias. Therefore, formal drug development requires an early screening of whether the potential drug candidates bear any activities on the hERG channels<sup>11</sup>. However, drug effects on cardiac repolarization cannot be accurately estimated by measuring hERG channel currents alone<sup>12</sup>, since drug responses on native  $I_{Kr}$  channel and action potential can be different from those measured in hERG.

In the present study cannabidiol (CBD), tetrahydrocannabinol (THC) and 11-nor-9-carboxy-tetrahydrocannabinol (11-nor-9-carboxy-THC), the main metabolite of THC was assessed for their effects on the hERG channels in an in vitro assay. CBD and THC are the major components of cannabis products for medicinal and recreational use, respectively, and since the latter is quickly metabolized to 11-nor-9-carboxy-THC, these three compounds were chosen to be tested in vitro.

Therefore, the aim of our work was to study the in vitro and in vivo effects of CBD a major cannabinoid on cardiac ventricular action potential, on ECG parameters, on the hERG and on other native cardiac transmembrane channels to provide experimental data for the elucidation of their possible adverse cardiac electrophysiological effects.

## Results

As our first test shown in Fig. 1, CBD was found to be an inhibitor of the hERG potassium channel with intermediate potency represented by  $IC_{50}$  values of  $2.07 \pm 0.12 \mu\text{M}$  ( $n = 6$ ) at room temperature. The  $IC_{50}$  values for the inhibition elicited by THC were higher ( $10.30 \pm 0.55 \mu\text{M}$ ,  $n = 6$  at room temperature). 11-Nor-9-carboxy-THC exhibited only a marginal effect ( $IC_{50} = 65.40 \pm 3.82 \mu\text{M}$ ,  $n = 4$  at room temperature).

The cardiac cellular electrophysiological effect of the most potent cannabis compound CBD was further studied on various transmembrane ionic currents by the whole-cell configuration of the patch clamp technique in native rabbit ventricular myocytes and on action potentials in rabbit and guinea pig right ventricular papillary muscle by the conventional microelectrode technique and on in vivo ECG studies in anaesthetized guinea pigs. Figure 2 shows that CBD lengthens action potential duration ( $APD_{90}$ ) slightly but significantly at  $1 \mu\text{M}$  and at  $2.5 \mu\text{M}$ . This latter effect depended on the stimulation frequency and vanished at slow pacing rate. At high  $10 \mu\text{M}$  concentration CBD exerted variable effect on repolarization including minimal or no change, shortening and lengthening of  $APD_{90}$  resulting statistically not significant alteration of APD. At  $1$  and  $2.5 \mu\text{M}$  CBD caused triangulation in some experiments but not in others reflected as not significant change in  $APD_{90}$ – $APD_{25}$ . Similar results were obtained in guinea pig papillary muscles where  $2.5$  and  $5 \mu\text{M}$  CBD increased  $APD_{90}$  from  $186.2 \pm 6.1$  ms and  $179.9 \pm 6.0$  ms to  $192.2 \pm 6.8$  and to  $191.5 \pm 8.9$  ms, respectively ( $p < 0.05$ ,  $n = 5$ ).

In anaesthetized in vivo guinea-pig experiments intravenous administration of  $0.3$  mg/kg and  $1$  mg/kg CBD lengthened QTc and QRS intervals in a statistically significant manner without significantly changing other ECG parameter (Fig. 3).

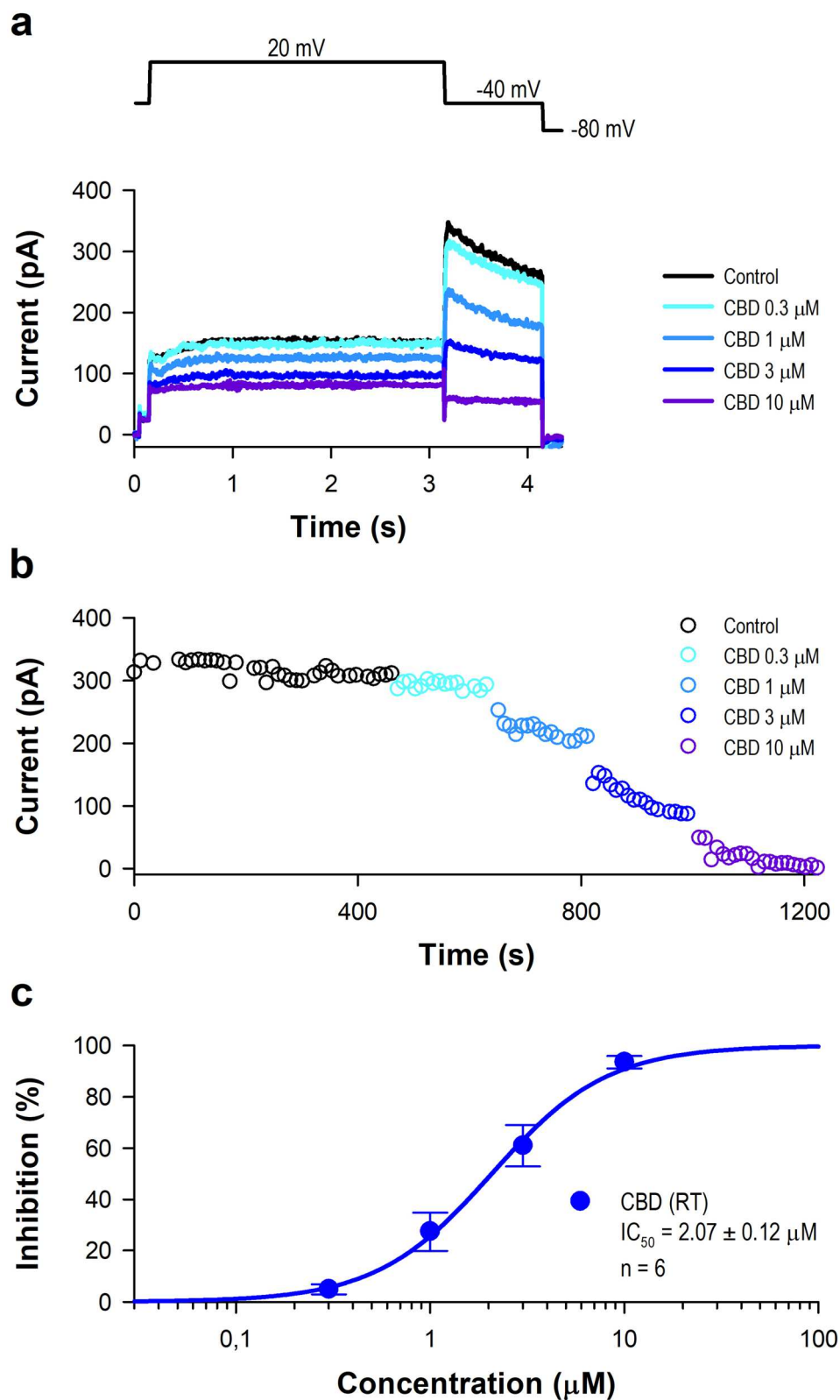
Whole-cell patch clamp experiments on rabbit native cardiac ventricular myocytes revealed significant and voltage-dependent inhibition of the rapid delayed rectifier potassium current ( $I_{Kr}$ ) (Figs. 4a,c) with an estimated  $IC_{50}$  value of  $6.5 \mu\text{M}$  after a  $20$  mV  $1$  s long test pulse and measured at  $-40$  mV as deactivating tail current (Fig. 4b).

The observation that high ( $10 \mu\text{M}$ ) concentration of CBD did not further lengthened APD prompted us to study the possible effect of CBD on inward L-type  $\text{Ca}^{2+}$  ( $I_{CaL}$ ) and late inward  $\text{Na}^{+}$  ( $I_{NaL}$ ) currents. As Fig. 5a,b show,  $10 \mu\text{M}$  CBD decreased  $I_{CaL}$  significantly and in a frequency-dependent manner. In addition,  $10 \mu\text{M}$  CBD also significantly inhibited  $I_{NaL}$  by  $41.5\%$  at  $-20$  mV (Fig. 5c,d).

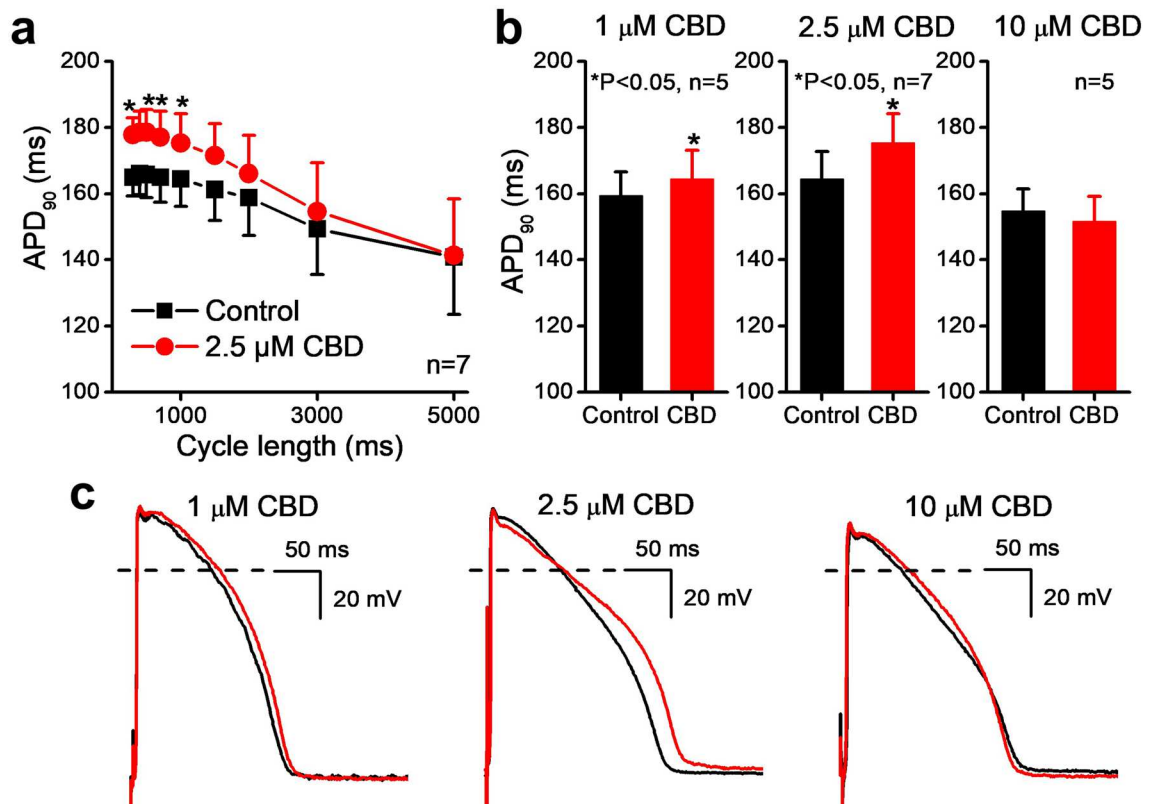
## Discussion

The main result of our study is that CBD lengthens repolarization at low and does not change it statistically significant manner at higher concentrations. This effect on repolarization in rabbit papillary muscle can be best explained by the multiple ion channel effects of CBD. Accordingly, at lower concentrations ( $1$ ,  $2.5$  and  $5 \mu\text{M}$ )  $I_{Kr}$  depression results in lengthening of  $APD_{90}$ , which is counterbalanced by inward  $\text{Ca}^{2+}$  and  $\text{Na}^{+}$  currents inhibition at  $10 \mu\text{M}$ . Similar effect was earlier described by quinidine, an antiarrhythmic drug, with reported proarrhythmic property<sup>13,14</sup>. The  $I_{Kr}$  inhibition by CBD, which is consistent with the hERG blockade, is most probably a direct effect on the channel but the effect of CBD on  $I_{NaL}$  and on  $I_{CaL}$  can be either direct or receptor mediated, as well. It needs more research to be established.

The higher  $IC_{50}$  values of THC and 11-nor-9-carboxy-THC than that of CBD in hERG current measurement in our study does not necessarily mean that CBD is more potent in other type of experiments since potency can



**Figure 1.** Effect of CBD on hERG current at room temperature. **(a)** Representative current curves obtained from HEK-hERG cells treated with 0.3, 1, 3, and 10  $\mu\text{M}$  CBD. The currents were recorded using the voltage protocol shown at the top of the panel after 3–5 min acute superfusion of the drugs without washout. **(b)** Time-course of the hERG peak tail current amplitude upon the application of different concentrations of CBD. **(c)** Dose–response curves of CBD's inhibitory activity on the hERG channel.

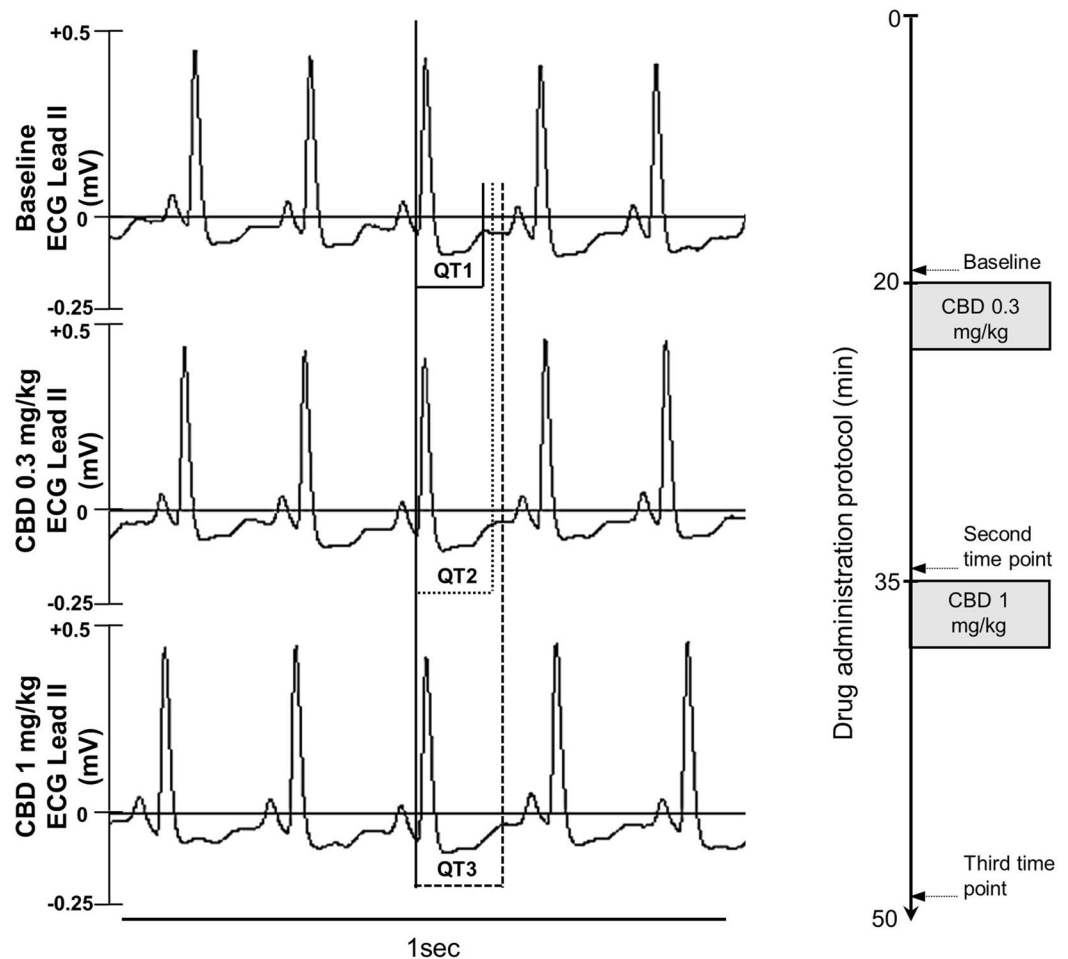


**Figure 2.** Effect of CBD after 30–50 min acute superfusion of the drug without washout on action potentials recorded from rabbit right ventricular papillary muscle at 37 °C. **(a)** The cycle length-dependent effect of 2.5 μM CBD on the duration of action potentials (APD<sub>90</sub>). Bar diagrams **(b)** indicates the effects of 1 μM, 2.5 μM and 10 μM CBD on the action potential duration during steady-state at 1000 ms cycle length. Original action potential traces are shown on **(c)** recorded at 1000 ms cycle length in control conditions and in the presence of 1 μM, 2.5 μM and 10 μM CBD.

differ from a target to another<sup>12</sup>. However, in our study due to technical limitations we focused our investigations to examine the effects of CBD in more depth.

Some previous study with cannabinoids showed effects on various transmembrane ion channels such as inward sodium<sup>9,15</sup>, inward calcium<sup>9</sup>, outward transient current<sup>16</sup> and human Kv1.5 and Kv4.3 channels<sup>10,17</sup>. Our results in rabbit ventricular myocytes are in good agreement with those reported by Al Kury et al. on inward calcium and sodium currents in rat ventricular myocytes<sup>9</sup>. In a previous study hERG channel inhibition and QT lengthening were also reported in anaesthetized rats<sup>18</sup> by a synthetic cannabinoid compound (JWH-030). This synthetic cannabinoid compound differs from those investigated by us and inhibited hERG channel with a relatively high IC<sub>50</sub> (88.36 μM). In addition, in rat ventricle hERG/I<sub>Kr</sub> seems not so important to control repolarization than Kv4.2 and Kv1.5 channels. Therefore, the cannabinoid-evoked QT changes in rat most likely can be attributed to Kv1.5 and Kv4.2 rather than hERG channel inhibition. It is worth to note that in the same study<sup>18</sup> a cannabinoid derivate JWH-030 did not change APD in low but shortened it at high (30 μM) concentration. Therefore, the results of our study is in partial agreement with these earlier reports and the differences are best explained by different preparations, chemical differences of the studied compounds and experimental conditions.

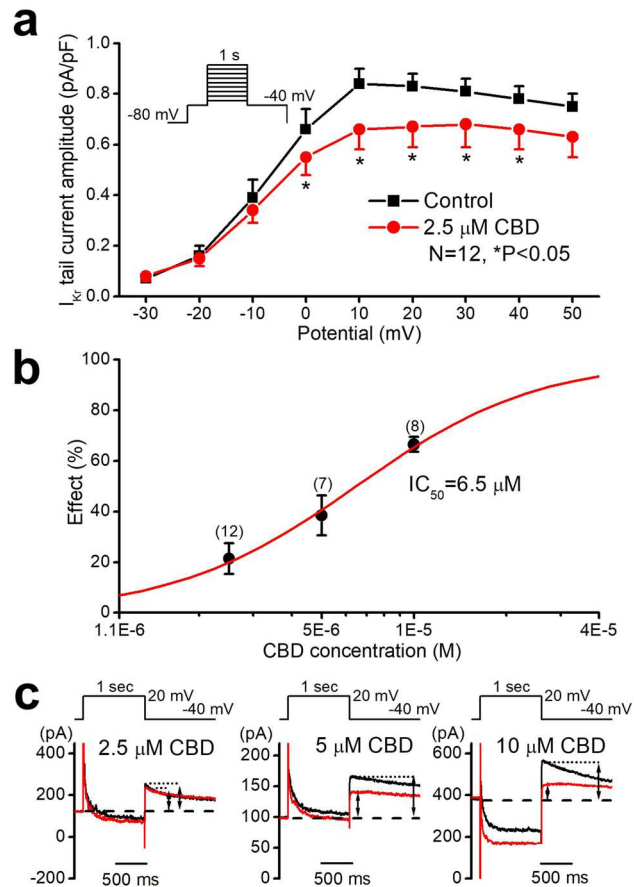
The IC<sub>50</sub> values reported here together with the C<sub>max</sub> values of CBD allow the assessment of cardiovascular risks of this compound. Based on the comparison of hERG or I<sub>Kr</sub> activity, cardiac action potential duration, and QT prolongation against QT effects and reports of arrhythmogenic (torsade de pointes) potential of 100 drugs, a margin of at least 30-fold between hERG IC<sub>50</sub> and C<sub>max</sub> was proposed to an acceptable degree of safety from arrhythmogenesis<sup>19</sup>. According to human pharmacokinetic data, the C<sub>max</sub> values for CBD might reach 0.35 μM and 0.58 μM after CBD smoking (19.2 mg) and oral intake (400 mg), respectively<sup>20</sup>. In our experiments CBD had an inhibitory effect on both the hERG channel and I<sub>Kr</sub> activity, with an IC<sub>50</sub> value higher than literary C<sub>max</sub> values in patients. Considering the IC<sub>50</sub> values for I<sub>Kr</sub> and hERG channel inhibition in our experiments (6.5 and 2.07 μM, respectively), the ratios of IC<sub>50</sub> and C<sub>max</sub> values are in the range of 3.57–18.57. This safety margin (below 30) suggests a potential proarrhythmic risk in human setting. However, previous clinical reports documented no significant QT and QT<sub>c</sub> prolongation in patients after CBD administration<sup>21</sup>. Also, in another study it was found that long term Sativex (CBD + THC) treatment evoked T wave changes only 1 out of 146 patients<sup>22</sup>. This might be explained by the effects of CBD on other ion channels than hERG and I<sub>Kr</sub> (eg. I<sub>CaL</sub> and I<sub>NaL</sub>). However, in patients who have slower drug elimination due to certain diseases or in case of concurrent use of medicines inhibiting the metabolism of CBD, higher C<sub>max</sub> values may develop and the risk of arrhythmia might be increased<sup>23</sup>. Moreover, when co-administered with pharmacological agents affecting cardiac repolarization, as well as in certain



(n=5)	RR (ms)	HR (bpm)	QT (ms)	QTc (ms)	QRS (ms)
<b>1. Baseline</b>	212.8±12.2	285.5±14.0	130.6±8.2	145.8±3.9	47.6±2.9
<b>2. CBD 0.3 mg/kg</b>	211.2±6.7	286.5±8.0	135.1±5.6	150.9±3.0*	48.1±2.4
<b>3. CBD 1 mg/kg</b>	204.9±5.6	295.7±7.1	139.4±5.3	157.8±3.1*	50.1±1.9*

**Figure 3.** The volume conducted electrocardiogram (ECG lead II) signals in regular sinus rhythm in a pentobarbital anaesthetized (30 mg/kg i.p. bolus injection) guinea pig at three different time points indicated with dashed arrows: **1.** drug-free baseline, value determined from 40 consecutive beats before drug administration; **2.** value determined from 40 consecutive beats 15 min after the 0.3 mg/kg intravenously (iv) administered cannabidiol (CBD) by 2 min bolus; **3.** value determined from 40 consecutive beats 15 min after the 1 mg/kg iv administered CBD by 2 min bolus. RR interval: the time elapsed between two successive R-waves of the QRS signal on the ECG. HR heart rate, QT interval the time from the start of the Q wave to the end of the T wave, QTc interval heart rate corrected QT interval, calculated with a correction method described earlier<sup>27,28</sup>, QRS interval the time from the onset to the end of the QRS complex. Table shows the mean ± SE values of the ECG intervals at three different time points. Changes in mean scores over three time points were compared using the repeated measures ANOVA with Bonferroni correction. \*p < 0.05 was taken as indicative of a statistically significant difference between values.

pathophysiological conditions such as hypokalaemia, or diseases like LQT syndrome, HCM, diabetes mellitus or heart failure where cardiac repolarization reserve or drug metabolism is impaired, CBD may have an additive effect, further increasing the proarrhythmic risk and the possibility of sudden cardiac death. Such additive



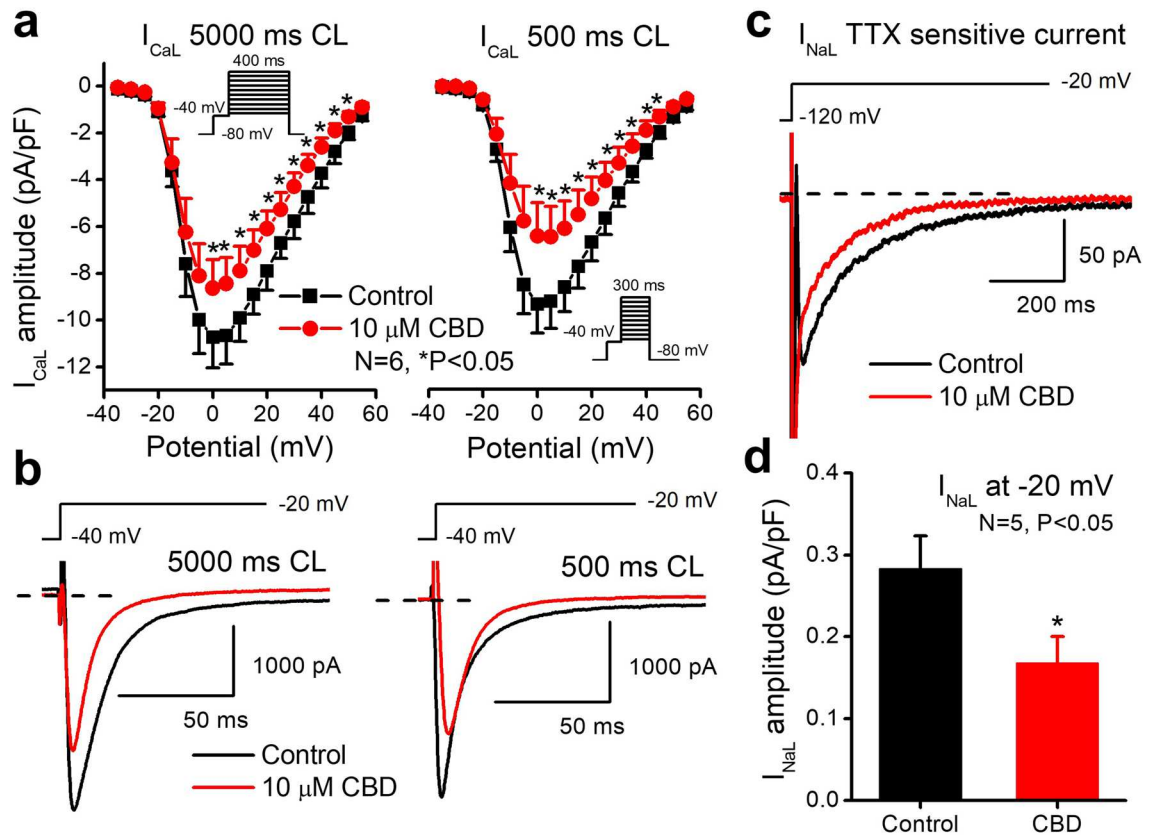
**Figure 4.** Effect of CBD after 3–5 min acute superfusion of the drug without washout on the rapid delayed rectifier potassium current ( $I_{Kr}$ ) in rabbit left ventricular myocytes at 37 °C. Current–voltage curves show the inhibition of  $I_{Kr}$  by 2.5  $\mu$ M CBD (**a**). (**b**) Displays CBD concentration–response curve indicating an estimated  $IC_{50}$  value of 6.5  $\mu$ M for  $I_{Kr}$  blockade. Original  $I_{Kr}$  current traces are shown in (**c**) in control conditions and in the presence of 2.5  $\mu$ M, 5  $\mu$ M and 10  $\mu$ M CBD recorded from rabbit left ventricular myocytes after a 1 s long pulse to 20 mV test potential with pulsing cycle length of 20 s.  $I_{Kr}$  deactivating tail current was measured at  $-40$  mV. The vertical axis on the left side of the panels shows the absolute current level. The dashed lines refer to the baseline for  $I_{Kr}$  tail current level after the test pulse at  $-40$  mV. The arrows indicate the amplitudes of the  $I_{Kr}$  tail currents.

effect was reported in both in experiments<sup>24</sup> and in patients<sup>25</sup>. The cardiovascular effects of CBD may only partly be interpreted on its effects on hERG and  $I_{Kr}$  ion channels, the cardiovascular safety of this compound may be influenced by its activities on other ion channels. Further studies are needed to assess the effects of other cannabinoids as well, and the in vivo relevance of these results, with special focus on the benefit–risk assessment of products with different cannabinoid content.

## Methods

The hERG channel current was measured by planar technology in HEK 293 cell line by the whole-cell configuration using an automated patch clamp system (Patchliner, Nanion Technologies GmbH., Munich, Germany) at room temperature as described previously<sup>12</sup>. The following solutions were used during automated patch-clamp recording (compositions in mM): internal solution: KCl 50, NaCl 10, KF 60, EGTA 20, HEPES 10, pH 7.2 (KOH); external solution: NaCl 140, KCl 4, glucose-monohydrate 5,  $MgCl_2$  1,  $CaCl_2$  3, HEPES 10, pH 7.4 (NaOH). The voltage protocol for hERG ion channel started with a short (100 ms)  $-40$  mV step to establish the baseline region. A depolarizing step was applied to the test potential of 20 mV for 3 s, and then the cell was repolarized to  $-40$  mV (1 s) to evoke outward tail current. The peak tail current was corrected the leak current defined during the first period to  $-40$  mV. Holding potential was  $-80$  mV. The pulse frequency was approximately 0.1 Hz. Recording started in external solution. After this control period, increasing concentrations of the test compound were applied, in order to record a complete concentration–response curve.

The action potential measurements were carried out in rabbit and guinea pig right ventricular papillary muscles by the conventional microelectrode techniques at 37 °C as described in detail earlier<sup>12,26</sup>. Isolated muscle preparations obtained from the right ventricle were individually mounted in a tissue chamber while superfused with oxygenated modified Locke's solution containing (in mM): NaCl 128.3, KCl 4,  $CaCl_2$  1.8,  $MgCl_2$  0.42,



**Figure 5.** Effect of CBD after 3–5 min acute superfusion of the drug without washout on L-type calcium ( $I_{CaL}$ ) and on the late sodium ( $I_{NaL}$ ) currents in rabbit left ventricular myocytes at 37 °C. On (a) current–voltage curves show the inhibition of  $I_{CaL}$  by 10  $\mu$ M CBD at 5000 ms (left) and at 500 ms (right) cycle lengths. Original  $I_{CaL}$  current traces are shown on (b) in control conditions and in the presence of 10  $\mu$ M CBD recorded from rabbit left ventricular myocytes at 5000 ms (left) and at 500 ms (right) cycle lengths at 0 mV test potential. TTX sensitive current ( $I_{NaL}$ ) traces (c) and a bar diagram (d) show the inhibition of  $I_{NaL}$  by 10  $\mu$ M CBD measured at –20 mV in rabbit left ventricular myocytes.

NaHCO<sub>3</sub> 21.4 and glucose 10 (pH 7.35–7.4) and stimulated through a pair of platinum electrodes with constant cycle length of 1000 ms. In case of cycle length-dependent measurements stimulation with different constant cycle lengths ranging from 300 to 5000 ms were also applied. Transmembrane potentials were recorded using conventional glass microelectrodes, filled with 3 M KCl and having tip resistances of 5–20 M $\Omega$ , connected to the input of a high impedance electrometer (Experimetria, type 309, Budapest, Hungary). The analog action potential signals were digitized with analogue-to-digital converters (ADA 3300, Real Time Devices Inc., State College, PA, USA) under software control (APES home-made software).

Transmembrane ion currents in native rabbit ventricular myocytes were measured by the whole-cell configuration of the patch clamp technique at 37 °C (Axopatch 200B, Molecular Devices Inc., Sunnyvale, CA, USA) as described in detail earlier<sup>12</sup>. Rapid delayed rectifier potassium current ( $I_{Kr}$ ), was recorded in HEPES-buffered Tyrode's solution containing (in mM) NaCl 144, NaH<sub>2</sub>PO<sub>4</sub> 0.33, KCl 4.0, CaCl<sub>2</sub> 1.8, MgCl<sub>2</sub> 0.53, glucose 5.5 and HEPES 5.0, at pH of 7.4. The composition of the pipette solution (in mM) was the following: KOH 110, KCl 40, K<sub>2</sub>ATP 5, MgCl<sub>2</sub> 5, EGTA 5, and HEPES 10 (pH was adjusted to 7.2 by aspartic acid). 1  $\mu$ M nisoldipine and 0.5  $\mu$ M HMR-1556 (the selective blocker of the slow delayed rectifier K<sup>+</sup> current— $I_{Ks}$ ) were added to the external solution to eliminate  $I_{CaL}$  and  $I_{Ks}$ , respectively.  $I_{Kr}$  was determined as tail current at –40 mV after the end of 1 s long depolarizing pulses ranging from –30 to +50 mV with pulsing cycle length of 20 s. The L-type calcium current ( $I_{CaL}$ ) was recorded in HEPES-buffered Tyrode's solution supplemented with 3 mM 4-aminopyridine. A special solution was used to fill the micropipettes (composition in mM: CsCl 125, TEACl 20, MgATP 5, EGTA 10, HEPES 10, pH was adjusted to 7.2 by CsOH).  $I_{CaL}$  current was evoked by 400 ms long depolarizing voltage pulses to various test potentials ranging from –35 to +55 mV with pulsing cycle length of 5 s. The holding potential was –80 mV. A short prepulse to –40 mV served to inactivate Na<sup>+</sup> current. The sodium current was activated by 2 s long depolarizing voltage pulses to –20 mV from the holding potential of –120 mV with pulsing cycle length of 5 s. After 5–7 min incubation with CBD the external solution was replaced by that containing 20  $\mu$ M TTX. TTX at this concentration completely blocks the late sodium current ( $I_{NaL}$ ). The external solution was HEPES-buffered Tyrode's solution supplemented with 1  $\mu$ M nisoldipine, 0.5  $\mu$ M HMR-1556 and 0.1  $\mu$ M dofetilide in order to block  $I_{CaL}$ ,  $I_{Ks}$  and  $I_{Kr}$  currents. The composition of the pipette solution (in mM) was: KOH 110, KCl 40, K<sub>2</sub>ATP 5, MgCl<sub>2</sub> 5, EGTA 5, HEPES 10 (pH was adjusted to 7.2 by aspartic acid).

ECG recordings were taken from adult guinea-pigs of both sexes (600–800 g) anaesthetized by intraperitoneal 30 mg/kg pentobarbital and I–III leads were recorded after 15 min of cumulative intravenous administration of CBD into the jugular vein<sup>27</sup>.

**Statistics.** All data are expressed as means  $\pm$  SEM. Statistical analysis was performed with Student's *t* test for paired data. The results were considered statistically significant when *p* was  $< 0.05$ .

**Animal ethics statement.** All experiments performed in rabbit and guinea pig ventricular preparations were carried out in compliance with the Guide for the Care and Use of Laboratory Animals (USA NIH publication NO 85-23, revised 1996) and conformed to the Directive 2010/63/EU of the European Parliament. The protocols have been approved by the Ethical Committee for the Protection of Animals in Research of the University of Szeged, Szeged, Hungary (approval number: I-74-24-2017) and by the Department of Animal Health and Food Control of the Ministry of Agriculture and Rural Development (authority approval number XIII/3331/2017).

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## Author contributions

D.C., B.C.-L., L.V. and A.V. conceived the experiment, P.O., B.P., L.T., A.P., Á.B., P.G., J.P. and E.T.-M. conducted the experiment(s), P.O., L.V. and A.V. analysed the results, D.C., T.K., J.H., L.V. and A.V. prepared the manuscript. All authors reviewed the manuscript.

## Competing interests

The authors declare no competing interests.

## Additional information

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