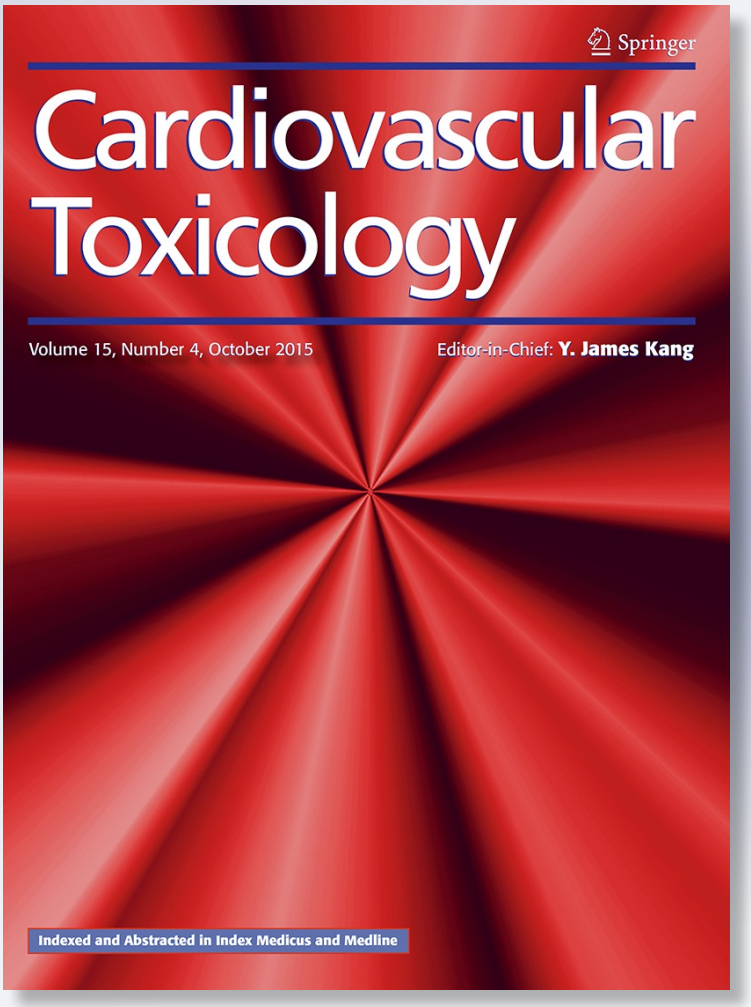
Cardiotoxic Electrophysiological Effects of the Herbicide Roundup® in Rat and Rabbit Ventricular Myocardium In Vitro

Steeve Gress, Sandrine Lemoine, Paolo-Emilio Puddu, Gilles-Eric Séralini & René Rouet

Cardiovascular Toxicology

ISSN 1530-7905
Volume 15
Number 4

Cardiovasc Toxicol (2015) 15:324-335
DOI 10.1007/s12012-014-9299-2



Springer

Cardiovasc Toxicol (2015) 15:324–335
DOI 10.1007/s12012-014-9299-2

Cardiotoxic Electrophysiological Effects of the Herbicide Roundup® in Rat and Rabbit Ventricular Myocardium In Vitro

Steeve Gress · Sandrine Lemoine · Paolo-Emilio Puddu · Gilles-Eric Séralini · René Rouet

Published online: 2 December 2014

Abstract Roundup (R), a glyphosate (G)-based herbicide (GBH), containing unknown adjuvants is widely dispersed around the world. Used principally by farmers, intoxications have increasingly been reported. We have studied R effects (containing 36 % of G) on right ventricular tissues (male Sprague–Dawley rats, up to 20,000 ppm and female New Zealand rabbits, at 25 and 50 ppm), to investigate R cardiac electrophysiological actions in vitro. We tested the reduced Ca^{++} intracellular uptake mechanism as one potential cause of the electrical abnormalities after GBH superfusion, using the Na^{+}/K^{+}-ATPase inhibitor ouabain or the 1,4-dihydropyridine L-type calcium channel agonist BAY K 8644 which increases I_{Ca}. R concentrations were selected based on human blood ranges found after acute intoxication. The study showed dose-dependent V_{max}, APD_{50} and APD_{90} variations during 45 min of R superfusion. At the highest concentrations tested, there was a high incidence of conduction blocks, and 30-min washout with normal Tyrode solution did not restore excitability. We also observed an increased incidence of arrhythmias at different doses of R. Ouabain and BAY K 8644 prevented V_{max} decrease, APD_{90} increase and the cardiac inexcitability induced by R 50 ppm. Glyphosate alone (18 and 180 ppm) had no significant electrophysiological effects. Thus, the action potential prolonging effect of R pointing to I_{Ca} interference might explain both conduction blocks and proarrhythmia in vitro. These mechanisms may well be causative of QT prolongation, atrioventricular conduction blocks and arrhythmias in man after GBH acute intoxications as reported in retrospective hospital records.

Keywords Roundup · Glyphosate · Action potential duration · Conduction blocks · Pro-arrhythmia · Calcium

S. Gress · G.-E. Séralini
Institute of Biology, Risk Pole, MRSH-CNRS, EA 2608 Estrogen, Reproduction and Cancer, University of Caen, Esplanade de la Paix, 14032 Caen Cedex, France

S. Gress · S. Lemoine · R. Rouet
Institute of Biology, EA 4650 Signalisation, électrophysiologie et imagerie des lésions d'ischémie-reperfusion myocardique, University of Caen, Esplanade de la Paix, 14032 Caen Cedex, France

S. Lemoine
Department of Anesthesiology and Critical Care Medicine, University Hospital of Caen, 14033 Caen Cedex, France

S. Lemoine
Faculty of Medicine, University of Caen, Caen, France

P.-E. Puddu (✉)
Laboratory of Biotechnologies Applied to Cardiovascular Medicine, Department of Cardiovascular Sciences, Respiratory, Nephrological, Anesthesiological and Geriatric Sciences, Sapienza University of Rome, Viale del Policlinico, 155, 00161 Rome, Italy
e-mail: paoloemilio.puddu@uniroma1.it

Introduction

Exposure of human and mammalian populations to environmental and industrial contaminants represents a growing concern due to the impact of these pollutants on human health [1]. Roundup (R), a glyphosate (G)-based herbicide (GBH), containing adjuvants such as polyoxy ethyl amine (POEA) is the most used in the world. Although several commercial formulations exist, only a few specifically declare their adjuvant contents [2]. R residues are found in tap water, food or feed as adjuvants or other active ingredients are found in ground water, to

the extent that where the study of health effects are concerned, an exposure to diluted whole formulation may be more representative of environmental pollution than just G exposure [3–7].

In the developing world, the main health problems arising from pesticides were described after accidental intoxications or by ingestion in suicidal cases. In these latter cases, pesticides were demonstrated to cause severe circulatory failure in poisoned humans [8–12]. Death was strongly associated with greater age, larger ingestion and high plasma G concentrations on admission (>734 ppm). Extreme exposure (around 100–200 ml of the pure formulation ingested) resulted in respiratory, heart and hepato-renal damage. In cases of suicide attempts, up to 500 ml were ingested [13]. In acute intoxication, G blood concentrations were 61 and 4,146 mg/l, respectively, in mild to moderate and fatal cases [14]. In 1973, the World Health Organization (WHO) estimated that 500,000 cases of serious acute pesticide poisoning occurred annually, whereas in 1985, there were 3 million cases of hospitalization and 220,000 deaths [8, 9]. There could be as many as 25 million agricultural workers in the developing world who suffer an episode of poisoning each year [15]. QT interval prolongation, a surface electrocardiographic index of action potential duration dispersion, atrioventricular conduction blocks and arrhythmias are very common after GBH intoxication [10, 12, 14]. The adjuvants of herbicides might have also contributed to cardiovascular disorders [14, 16–18].

The present investigation was undertaken to assess R cardiac electrophysiological effects in vitro, which represents the first ever attempt in mammals and a potential suggestion for further research in humans aimed at understanding the toxicological consequences on the heart. Importantly, arrhythmia occurrence may be explained by the effects of superfused agents and by analyzing action potential characteristics [19]. Dose–effect relationships of R on electrophysiological parameters and the occurrence of arrhythmias and conduction blocks were studied after carefully considering reports of human blood concentrations of G after mild to moderate and fatal intoxications [14]. We also tried to test whether a reduced Ca^{++} intracellular uptake mechanism might be a potential cause of the electrical abnormalities after GBH superfusion [20], by both indirect and direct increases of intracellular Ca^{++} using the Na^+/K^+-ATPase inhibitor ouabain acting, among other mechanisms, through Na_i^+ increase [21] or the 1,4-dihydropyridine L-type calcium channel agonist BAY K 8644 which stimulates the I_{Ca} [22]. Moreover, we tested the electrophysiological effects of G (18 and 180 ppm contained as active principles in R 50 and 500 ppm, respectively), in order to determine whether the adjuvant itself or G was responsible for the overall cardiovascular effects of R.

Materials and Methods

Ethics

Care of the animals complied with the recommendations of the Helsinki Declaration, and the study was performed in accordance with the regulations of the official edict of the French Ministry of Agriculture (A14-118-004). Sprague–Dawley male rats weighing 260–280 g, all 60 days old, and New Zealand white female rabbits weighing 1.8–2.2 kg, all around 70 days old were euthanized under anesthesia with sodium pentobarbital 125 mg/kg i.p. Species were selected based on the idea that rats are commonly used to investigate toxicity effects [23], whereas rabbits may more closely represent human electrophysiology in vitro [24, 25]. In this study, fewer concentrations (yet similar species-dependently) were studied in rabbits, than in rats in order to spare the former species in accordance with the prevailing ethical imperative for experimental research. As rabbits present less arrhythmias than rats, we chose to use male rats and female rabbits to maximize, species-specifically, the respective incidences of arrhythmias [26, 27].

The hearts were quickly removed after thoracotomy and placed in a cardioplegic solution at room temperature for 30 min. A thin standard longitudinal strip (10 × 5 mm for rats and 16 × 8 mm for rabbits) of the right ventricular free wall was pinned, endocardial surface upward, in a perfusion chamber (volume of 3 ml). The ventricular strip was superfused at the rate of 3 ml/min with cardioplegic and normal Tyrode solution. Temperature was controlled and maintained at 36.5 ± 0.5 °C by a circulating thermostat-controlled bath (Polystat 5HP, Bioblock, Illkirch, France).

Superfusion Solutions

Normal Tyrode solution was oxygenated with 95 % O_2 and 5 % CO_2 and kept at 36.5 ± 0.5 °C. The composition of the Tyrode solution was in mmol/l: Na^+ 135, K^+ 4, Ca^{++} 1.8, Mg^{++} 1, $H_2PO_4^-$ 1.8, HCO_3^- 25, Cl^- 117.8 and glucose 11. The pH was 7.39 ± 0.05. The cardioplegic solution used for dissection and stabilization differed from normal Tyrode solution with higher glucose (from 11 to 55 mmol/l) in order to provide energy and hyperkalemia (from 4 to 30 mmol/l) to depolarize cardiac cells.

The choice of the concentrations of ouabain and BAY K 8846 and the procedures used to dilute them were based and/or followed those of previous studies of each agent alone on rat heart, in vitro [21, 28].

Chemicals

The herbicide R Ultra containing 36 % of acid G (R, ISO 9002, corresponding to 100 %) is a commercial formulation freely sold in France. The R was diluted in Tyrode solution at 2.5, 25, 50, 500, 5,000 and 20,000 ppm of R.

Other chemicals, glyphosate, ouabain and BAY K 8644 were purchased from Sigma-Aldrich (Saint Quentin Fallavier, France). Carbogen O_2/CO_2 95 %/5 % was purchased from Air Liquide Healthcare (France).

Data Acquisition and Analysis

The myocardial strips were stimulated at a frequency of 2 Hz for rats and 1 Hz for rabbits via a bipolar silicon-coated steel wire electrode. Rectangular pulses of 2 ms in duration and twice diastolic threshold intensity (around 2–2.5 V) were delivered by a programmable stimulator (SMP 310, Biologic, Grenoble, France).

Transmembrane potentials were recorded by the use of intracellular glass microelectrodes pulled from borosilicate filament tubes (GC 200F-15; Phymep, France) on a single-barreled microelectrode puller (Narashige, distributed by OSI, France). Microelectrodes were filled with KCl 3 mol/l (tip resistance ranging from 10 to 30 MΩ) and coupled to Ag/AgCl microelectrode holders leading to a home-built double-input stage of a high-impedance capacitance-neutralizing amplifier. The reference Ag/AgCl electrode was positioned in the superfusion chamber, close to the preparation. The recordings were displayed on a memory dual-beam storage oscilloscope (Gould Instrument Systems Inc, USA). The following cardiac action potential (AP) characteristics were automatically stored and measured by a system of cardiac AP automatic acquisition and processing device (DATAPAC, Biologic, Grenoble, France): resting membrane potential (RMP), AP amplitude (APA), AP duration at 50 % of repolarization (APD_{50}), AP duration at 90 % of repolarization (APD_{90}) and maximal upstroke velocity (V_{max}). During superfusion, conduction blocks and arrhythmias were recorded. Whenever possible, the same impalement was maintained throughout the experiment; however, when it was lost, readjustment was attempted. If the readjusted parameters deviated $\leq$10 % from the previous ones, experiments were continued; otherwise, they were discarded. The data were acquired though PowerLab System (4/26) controlled by LabChart (AD Instrument, UK).

Experimental Protocol

A 30-min cardioplegic perfusion was followed by a 120-min stabilization period with Tyrode solution. In separate groups, the preparations were superfused with a range of R (0–20,000 ppm) during 45 min. At the end of R superfusion, a 30-min washout superfusion period was performed with normal Tyrode solution (Fig. 1). This protocol was adopted for both rat and rabbit right ventricular muscles. The R was diluted in normal Tyrode solution. Each experiment was repeated ($n = 8$ for rats and $n = 6$ for rabbits), for the selected concentrations of R. Pharmacological studies with ouabain 25 µmol/l and BAY K 8644 200 nmol/l were started 15 min before superfusing

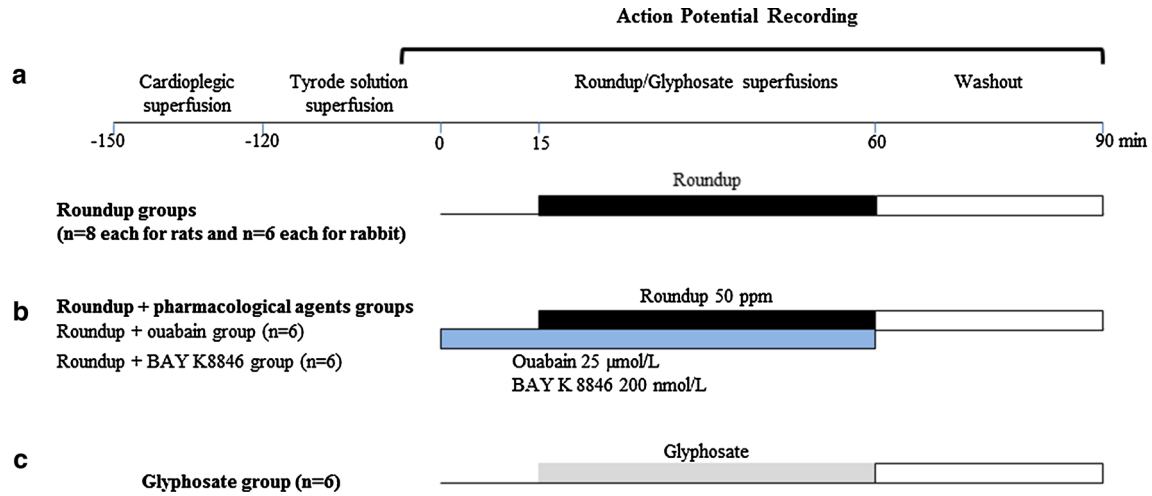


Fig. 1 Protocol. **a** There were 4 Roundup groups in rat ventricular tissues ($n = 8$ each), from 2.5 to 500 ppm, since at 5,000 and 20,000 ppm it was not possible to measure any electrical activity. Roundup at 0 ppm served as control group ($n = 4$ in rat). There were also two Roundup groups in rabbit ventricular tissue ($n = 6$ each), at 25 and 50 ppm. Roundup at 0 ppm served as control group ($n = 10$ in rabbit); **b** Roundup at 50 ppm was investigated concomitant with a previous 15 min superfusion of ouabain 25 µmol/l or BAY K 8644 200 nmol/l ($n = 6$ each); **c** Glyphosate 18 and 180 ppm were finally tested ($n = 6$ each) as these concentrations were those present with, respectively, Roundup 50 and 500 ppm

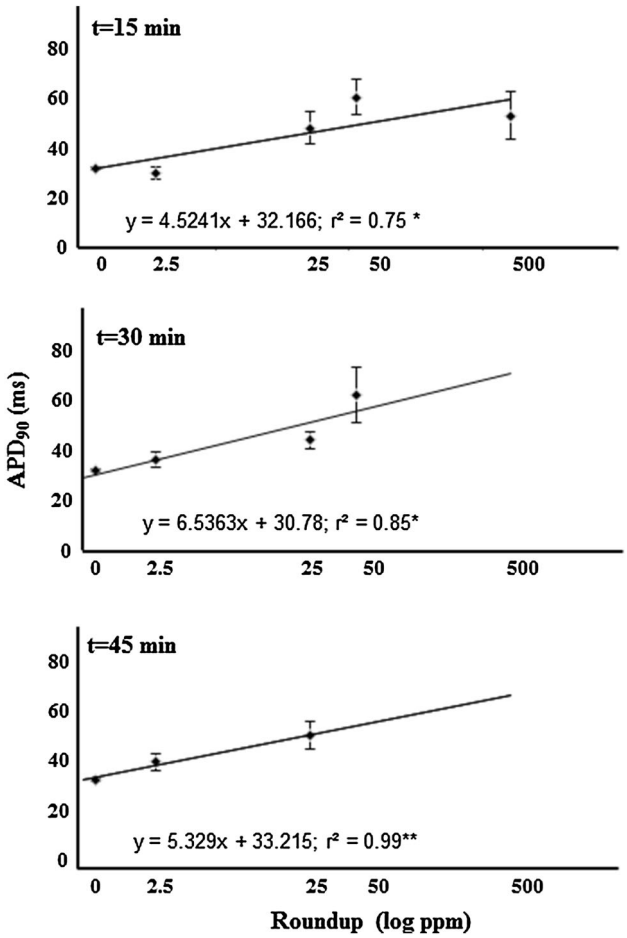


Fig. 2 Time- and concentration-dependent changes of APD_{90} after Roundup in rat ventricular tissues. Action potential duration at 90 of repolarization (APD_{90}) are presented at 15 (*upper panel*), 30 (*middle panel*) and 45 (lower panel) min of Roundup superfusion at 0 (control), 2.5, 25, 50 and 500 ppm. Values are mean ± SEM. $*p < 0.05$ and $**p < 0.01$ according to the critical values of *r*, Pearson product-moment correlation coefficient

R and were performed each on six rat preparations (Fig. 1). Finally, G 18 and 180 ppm were tested ($n = 6$ each) as these concentrations of G were those present in R 50 and 500 ppm, respectively (Fig. 1). There were time-related controls for both rat ($n = 4$) and rabbit ($n = 10$) ventricular tissues. In these experiments, electrophysiological acquisitions were obtained at time periods calculated in order to present adequate comparisons with respective experimental groups.

Statistical Analysis

Results were expressed as mean ± standard error of the mean (SEM). Significance of differences was calculated in absolute values. Statistical differences were determined by a Student's *t* test and for the simple linear regression and according to the critical values of Pearson Product-Moment

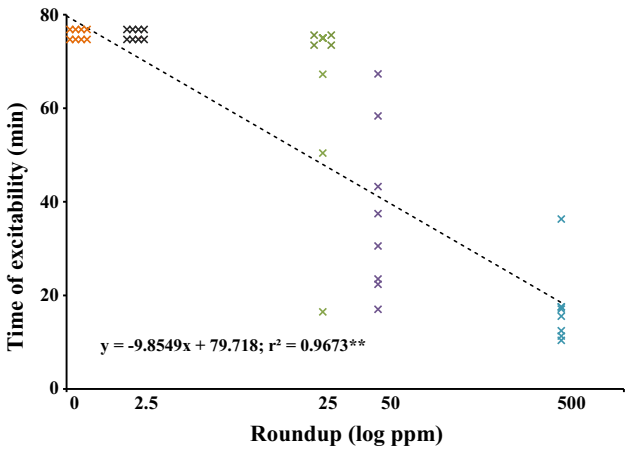


Fig. 3 Correlation between Roundup concentrations and inhibition of excitability in rat ventricular tissues. Occurrence of cardiac inexcitability (inexcitability occurs when stimulation was unable to obtain an action potential in the preparation) during the evolution of rat experiments. Crosses represent the experiments and indicate those developing inexcitability (thus, they remain excitable until then to stimulation) or remaining excitable until the end of the washout. Under the dose of 2.5 ppm, a normal excitability was recorded during all the experiments. $**p < 0.01$ according to the critical values of *r*, Pearson product-moment correlation coefficient

Correlation Coefficient (*r*). A *p* value <0.05 was considered statistically significant.

Results

Effects of Roundup on the Occurrence of Spontaneous Arrhythmias and Conduction Blocks

Rat tissues were, as expected, very sensitive to R [6, 29], and at 5,000 and 20,000 ppm, no electrical activity could be measured a few seconds after starting superfusions. We tested and recorded electrical activity on rat ventricular tissues from 0 to 500 ppm of R. Only 25 and 50 ppm were used in rabbit ventricular tissues. Depending on R concentration and superfusion duration, there were, in rat tissues, action potential lengthening (Fig. 2), a highly significant, dose-dependent decrease in excitability (Fig. 3: $r^2 > 0.96$), and the decrease in excitability induced, in fine, a high incidence of conduction blocks (Fig. 4; Table 1). Concomitantly, both in rat and rabbit ventricular tissues, there were salvos (spontaneous sustained arrhythmias, superior to ten spontaneous AP) recorded during ischemia and reperfusion periods and coded as present or absent with R superfusion (Fig. 5).

Rat Tissue Experiments

Superfusing R at 2.5 and 25 ppm increased the incidence of salvos, in comparison with the control group (Table 1). At

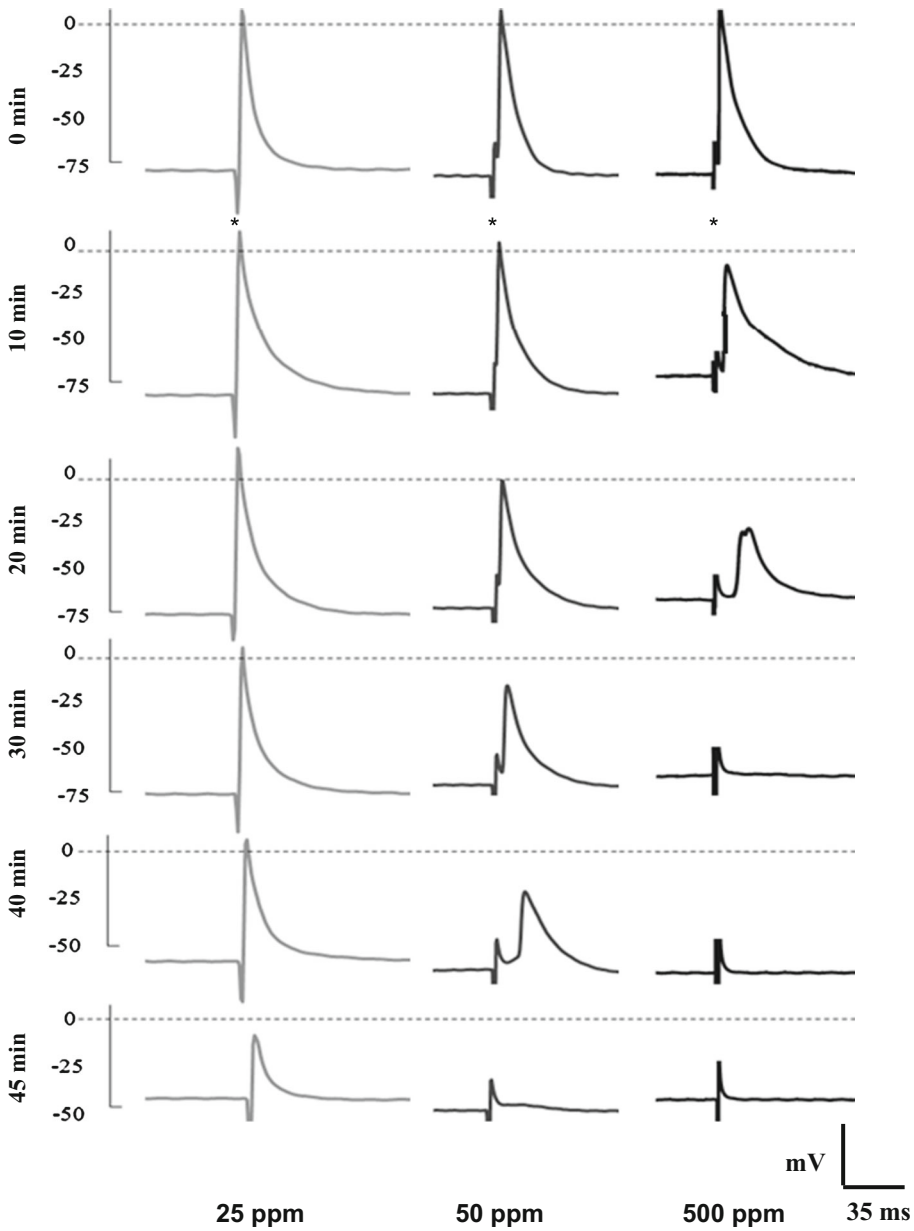


Fig. 4 Appearance of conduction blocks in rat ventricular tissues after Roundup superfusion. Roundup is superfused at 25, 50 and 500 ppm at 6 different times (0, 10, 20, 30, 40 and 45 min). Conduction block occurred for 50 (at 45 min) and 500 ppm (at 30 min). The *stars* (at 0 min) point to the stimulation artifact on each action potential. Voltage and time scales (35 ms) are also illustrated

higher concentrations (50 and 500 ppm), there was a decreased incidence of arrhythmias although action potential duration (APD) was even more prolonged (Table 2). Exposure to R significantly increased APD_{90}: +114 % after 30 min of R 50 ppm superfusion (from 34 ± 4 to 73 ± 11 ms, $p < 0.01$); and +65 % after 15 min of R 500 ppm superfusion (from 32 ± 2 to 53 ± 8, $p < 0.005$), as compared to baseline values (Table 2).

Rabbit Tissue Experiments

Superfusing R at 25 and 50 ppm induced salvos and ventricular tachycardia (Table 1; Fig. 5), and no conduction blocks were seen. Salvos were seen in 83 % of experiments at 25 and 50 ppm, whereas ventricular tachycardia was recorded in 50 and 67 % of experiments. There was substantial agreement between the two mammalian species investigated in the present study, apart from the conduction block seen in rat tissues which was not seen in rabbit tissues but arrhythmogenic effects were clearly shown in both rat and rabbit ventricular myocardial tissues.

Electrophysiological Effects of Roundup

The intracellular studies provided means to potentially explain the mechanisms whereby transmembrane ion conductances might have influenced the occurrence of repetitive responses or inhibition of excitability, in rat and rabbit

Table 1 Arrhythmias and conduction blocks recorded during Roundup superfusion in ventricular tissues

Doses of Roundup (ppm)	0	2.5	25	50	500
Rat					
Salvos	1	5	7	2	2
Ventricular tachycardia	0	1	3	2	1
Blocks	0	0	1	6	8
Rabbit					
Salvos	0	not done	5	5	not done
Ventricular tachycardia	0	not done	3	4	not done
Blocks	0	not done	0	0	not done

Roundup at 0 ppm served as control group for both rat ($n = 4$) and rabbit ($n = 10$) preparations. Both rat ($n = 8$ each concentration) and rabbit ($n = 6$ each concentration) experiments are indicated. Note that no experiment was performed in rabbit tissues at 2.5 and 500 ppm

ventricular tissues. In fact, V_{max} represents inward Na^+ current (with consistent changes in RMP and APA), whereas APD_{50} and APD_{90} reflect the balance of the inward current with outward K^+ [30].

Rat Tissue Experiments

Superfusing R at 50 and 500 ppm significantly modified the action potential parameters. As illustrated in Fig. 4 and Table 2: There was a significant depolarization (from baseline -78 ± 2 to -64 ± 4 mV after 30 min of R 50 ppm, $p < 0.01$), a V_{max} decrease (from baseline 259 ± 18 to 55 ± 27 V/s after 30 min of R 50 ppm, $p < 0.005$ and from baseline 277 ± 15 to 169 ± 46 V/s after 15 min of R 500 ppm, $p < 0.05$), decrease of APA (from baseline 103 ± 3 to 66 ± 14 mV after 30 min of R 50 ppm, $p < 0.005$ and from baseline 109 ± 5 to 66 ± 7 mV after 15 min of R 500 ppm, $p < 0.005$) and lengthening of APD_{50} (from baseline 10 ± 1 to 21 ± 5 ms after 30 min of R 50 ppm, $p < 0.01$) and APD_{90} (from baseline 34 ± 4 to 73 ± 11 ms after 30 min of R 50 ppm, $p < 0.01$ and from baseline 32 ± 2 to 53 ± 8 ms after 15 min of R 500 ppm, $p < 0.005$). As illustrated in Figs. 3 and 4, inexcitability of rat ventricular tissue occurred in all R experiments at 50 and 500 ppm and in three experiments after 25 ppm of R superfusion. There was a highly significant correlation between the R dose and the timing of inexcitability occurrence ($r^2 > 0.96$). Moreover, R washout did not restore electrical activity. Superfusion of the lowest R concentrations (2.5 and 25 ppm) induced significant effects on action potential parameters which were very similar but of lesser extent: RMP was significantly reduced, becoming less negative, whereas both APD_{50} and APD_{90} were significantly prolonged (Table 2). Again, washout of R superfusion did not modify APD_{90} lengthening (Fig. 4).

Rabbit Tissue Experiments

The directional changes of R at 25 and 50 ppm investigated in rabbit ventricular tissues were similar to those seen in rat tissues. There were changes of RMP, V_{max} and APA (although with some inconsistencies). However, the only statistically significant changes were those of action potential durations (both APD_{50} and APD_{90}), which were significantly prolonged (Table 3) and whose starting durations were expectedly much longer than those seen in rats. In rabbit ventricular tissue, due to absent conduction blocks, it was possible to fully obtain APD data at 45-min superfusion and APD_{50} was 86 ± 3–112 ± 16 ms of R 25 ppm, $p < 0.01$ and 95 ± 2–109 ± 7 ms of R 50 ppm, $p < 0.05$, whereas APD_{90} was 136 ± 3–164 ± 9 ms of R 25 ppm, $p < 0.005$ and 131 ± 2–153 ± 9 ms of R 50 ppm, $p < 0.005$ (Table 3). Also in rabbit tissues, R washout was not followed by APD restoration.

Effects of Ouabain and BAY K 8846 on Roundup 50 ppm Superfusion in Rat Ventricular Tissues

Only 50 ppm of R was considered for pharmacological studies as that was the lowest concentration where evident electrophysiological changes were seen in rat ventricular tissues. Ouabain 25 μmol/l and BAY K 8644 200 nmol/l, administered 15 min before and during R 50 ppm superfusion, prevented depolarization induced by 45 min of R 50 ppm superfusion (Fig. 6; Table 2). Compared with R 50 ppm superfusion alone, ouabain 25 μmol/l and BAY K 8644 200 nmol/l attenuated the V_{max} decrease ($p < 0.05$ vs. R 50 ppm; Fig. 6; Table 2). Ouabain 25 μmol/l and BAY K 8644 200 nmol/l prevented the reduction of APD_{50} and APD_{90} observed in the presence of R 50 ppm superfusion (at least, $p < 0.05$; Fig. 6; Table 2). Finally, both ouabain and BAY K 8644 prevented the occurrence of conduction blocks observed in response to 45 min of R 50 ppm superfusion (Fig. 7; Table 2).

Effects of Glyphosate Superfusion on Electrophysiological Parameters in Rat Ventricular Tissues

Superfusion of G (at both 18 and 180 ppm) did not substantially modify RPM, V_{max}, APA and APD_{50} and APD_{90} as compared to baseline values (Table 2). With G 180 ppm, there was a slight and significant reduction of APD_{90} at 30 min ($p < 0.05$). No dysrhythmia or conduction blocks were observed in the presence of G (18 and 180 ppm).

Table 2 Rat cardiac action potential parameters during Roundup (2.5 to 500 ppm), Glyphosate or pharmacological agents with Roundup 50 ppm superfusion

	Controls ($n = 4$)	Roundup (R) groups				Glyphosate (G) groups		Roundup + pharmacological groups	
		R (2.5 ppm) ($n = 8$)	R (25 ppm) ($n = 8$)	R (50 ppm) ($n = 8$)	R (500 ppm) (n=8)	G (18 ppm) ($n = 6$)	G (180 ppm) ($n = 6$)	Oua (25 µmol/l) + R (50 ppm) ($n = 6$)	BayK (200 nmol/l) + R (50 ppm) ($n = 6$)
RMP (mV)									
Initial	−78 ± 3	−79 ± 1	−78 ± 1	−78 ± 1	−78 ± 1	−83 ± 1	−85 ± 1	−84 ± 1	−84 ± 1
15	−78 ± 3	−75 ± 2	−70 ± 4**	−74 ± 4	−72 ± 3	−81 ± 2	−83 ± 2	−82 ± 3	−84 ± 2
30	−78 ± 2	−74 ± 2	−71 ± 2**	−64 ± 4**	CB	−83 ± 1	−85 ± 1	−84 ± 3	−81 ± 3
45	−80 ± 1	−79 ± 2	−66 ± 4***	CB	CB	−82 ± 1	−83 ± 2	−83 ± 2	−84 ± 3
V_{max} (V/s)									
Initial	295 ± 9	298 ± 19	190 ± 21	259 ± 18	277 ± 15	246 ± 12	261 ± 30	263 ± 20	265 ± 21
15	298 ± 28	254 ± 48	168 ± 27	145 ± 21**	169 ± 46*	261 ± 48	264 ± 45	200 ± 28	197 ± 41
30	296 ± 33	330 ± 3,442	154 ± 31	55 ± 27***	CB	268 ± 47	283 ± 58	231 ± 26*	171 ± 33**
45	290 ± 48	269 ± 79	193 ± 64	CB	CB	295 ± 53	263 ± 40	277 ± 37	252 ± 39
APA (mV)									
Initial	98 ± 2	112 ± 2	94 ± 3	103 ± 3	109 ± 5	98 ± 2	93 ± 3	100 ± 2	95 ± 2
15	96 ± 2	108 ± 7	91 ± 10	84 ± 7**	66 ± 7***	99 ± 4	93 ± 5	82 ± 3**	86 ± 3
30	98 ± 2	107 ± 7	89 ± 10	66 ± 14***	CB	100 ± 5	99 ± 3	77 ± 5**	85 ± 2*
45	98 ± 1	118 ± 6	89 ± 8	CB	CB	100 ± 4	98 ± 4	91 ± 5	91 ± 3
APD_{50} (ms)									
Initial	11 ± 2	12 ± 1	10 ± 1	10 ± 1	10 ± 1	10 ± 1	11 ± 1	9 ± 1	10 ± 1
15	9 ± 1	10 ± 1	13 ± 1*	17 ± 1***	12 ± 2	11 ± 1	11 ± 1	8 ± 1	10 ± 1
30	9 ± 1	9 ± 1	11 ± 1	21 ± 5**	CB	10 ± 2	10 ± 1	7 ± 1	11 ± 1
45	9 ± 1	8 ± 1	13 ± 1*	CB	CB	9 ± 1	9 ± 1	7 ± 1	11 ± 2
APD_{90} (ms)									
Initial	31 ± 2	30 ± 1	32 ± 2	34 ± 4	32 ± 2	33 ± 2	32 ± 1	31 ± 1	30 ± 1
15	29 ± 3	30 ± 2	38 ± 4*	61 ± 7**	53 ± 8***	32 ± 1	32 ± 1	30 ± 2	33 ± 2
30	32 ± 3	37 ± 3**	44 ± 3**	73 ± 11**	CB	33 ± 1	29 ± 1*	27 ± 1	34 ± 1
45	32 ± 3	40 ± 3***	50 ± 6***	CB	CB	33 ± 1	29 ± 1	28 ± 2	39 ± 3**

Initial values were not significantly different. There were $n = 6$ or 8 experiments for each group. Values are mean ± SEM

RMP: resting membrane potential; V_{max}: maximal upstroke velocity; APA: action potential amplitude; APD_{50}: action potential duration at 50 % of repolarization; APD_{90}: action potential duration at 90 % of repolarization; CB: conduction block

* $p < 0.05$; ** $p < 0.01$; *** $p < 0.005$ versus baseline values

Discussion

GBH ingested after suicide attempts or accidents resulted in a mortality rate of 2.6 and 1.8 %, respectively [12]. In GBH-poisoned persons, a high incidence of QTc interval prolongation and atrioventricular conduction blocks (from minimal to high grade) were reported along with arrhythmias, longer QTc and older age predicting mortality in multiple logistic regression analyses with excellent accuracy [12]. However, the exact mechanism of death and whether QT prolongation and heart blocks (with an incidence ranging from 27 to 72 %) after G-surfactant herbicide-poisoned intoxication [12] is still unknown. R Ultra used in the present investigation contains 36 % of G and 16 % of an unknown adjuvant [2]. Therefore, it was possible to test only R and G separately in the present study. However, future studies might certainly consider also POEA, especially POEA-15, although other adjuvants should also be considered [2]. In myocardial tissues, the concentrations to investigate might well be in the range up to 500 ppm, based on the observation of the present study that after 5,000 and 20,000 ppm, the tissues became immediately inexcitable, at least in rat preparations.

It was recently shown in rat testis and Sertoli cells that R disrupts male reproductive functions by triggering L-type voltage-dependent Ca^{++}-channel-mediated cell death [20].

Table 3 Rabbit cardiac action potential parameters during Roundup superfusion

Controls ($n = 10$)		Roundup (R) groups	
		R (25 ppm) ($n = 6$)	R (50 ppm) ($n = 6$)
RMP (mV)			
Initial	−82 ± 1	−83 ± 1	−82 ± 1
15	−82 ± 1	−82 ± 2	−84 ± 3
30	−82 ± 1	−83 ± 3	−80 ± 2
45	−83 ± 1	−81 ± 2	−84 ± 2
V_{max} (V/s)			
Initial	285 ± 23	256 ± 18	289 ± 22
15	243 ± 24	205 ± 33	228 ± 41
30	269 ± 27	227 ± 23	210 ± 11
45	223 ± 14	253 ± 56	252 ± 27
APA (mV)			
Initial	103 ± 3	116 ± 4	103 ± 1
15	102 ± 4	107 ± 4	115 ± 4
30	102 ± 3	109 ± 7	96 ± 2*
45	103 ± 2	107 ± 6	105 ± 2
APD_{50} (ms)			
Initial	104 ± 3	86 ± 3	95 ± 2
15	112 ± 7	100 ± 12*	100 ± 12
30	113 ± 6	119 ± 11**	118 ± 5*
45	119 ± 8	123 ± 16**	115 ± 5**
APD_{90} (ms)			
Initial	142 ± 3	136 ± 3	131 ± 2
15	147 ± 6	159 ± 21*	154 ± 13
30	151 ± 4	165 ± 9**	150 ± 11*
45	153 ± 7	171 ± 14**	153 ± 9***

There were $n = 10$ for controls and $n = 6$ experiments for each Roundup dose. Values are mean ± SEM

RMP: resting membrane potential; V_{max}: maximal upstroke velocity; APA: action potential amplitude; APD_{50}: action potential duration at 50 % of repolarization; APD_{90}: action potential duration at 90 % of repolarization. There is no significant difference between the different groups for the baseline values of each electrophysiological parameter

* $p < 0.05$; ** $p < 0.01$; *** $p < 0.005$ versus baseline values

In particular, it was shown that R at 360 ppm led to an important decrease in $^{45}Ca^{++}$ influx, whereas R at 36 ppm increased $^{45}Ca^{++}$ uptake, an effect ascribed to G and partially prevented by the L-type voltage-dependent Ca^{++}-channel antagonist nifedipine 10 µmol/l, thus indicating that disruption in Ca^{++} homeostasis plays a critical role in the toxic effects of GBH [20]. More than 20 years ago, it was demonstrated that G increased mitochondrial membrane permeability to protons and Ca^{++}, suggesting early on a mechanism for the toxic effect of GBH by a cationic chelator action [31]. These mechanistic hypotheses were specifically tested in the present investigation.

For the first time, in rat and rabbit heart ventricular tissues, we studied the effects of R on in vitro electrophysiology. G alone (at 18 and 180 ppm to simulate the G content of R at 50 and 500 ppm) was also investigated in rat ventricular tissues. Finally, a complementary study was performed in rat ventricular tissues, using pharmacological agents to increase L-type voltage-dependent Ca^{++}-current by BAY K 8644 [22] or Ca^{++} intracellular content by ouabain, a Na^+/K^+-ATPase inhibitor [21], before superfusing R at 50 ppm. Our results fit the hypothesis of decreased Ca^{++} uptake as the possible consequence of a cationic chelator action [20, 31] to explain R toxic effects, and we provide some electrophysiological evidence to further support it. Indeed, APD_{90} prolongation, conduction blocks, dose-dependent inexcitability and proarrhythmia may all follow an I_{Ca} decrease, thus accounting for lower effects of R 50 ppm when intracellular Ca^{++} is increased by the pharmacological approach tested. However, it is evident from the dose-dependent effects of G alone that the mechanism of R toxicity changes around the critical adjuvants concentration of the unknown surfactant and the latter should help intracellular penetration [2] of an otherwise hydrophilic G which is expected to show a much lower effect in a superfused tissue in vitro. When the exact chemical nature of the unknown adjuvant is disclosed [2], an investigation will be possible of its electrophysiological effects in myocardial tissues which should be done to elucidate the toxic effects of R, relevant to human poisoning [12]. Indeed, it would be important to know the exact nature of the adjuvant as also the mixture (of R plus the unknown adjuvant at present) might have electrophysiological effects, and these should be investigated.

Cardiac glycosides, such as ouabain, are useful clinically to increase inotropy of the failing heart [32] and are thought to act via inhibition of Na^+/K^+-ATPase activity, resulting in Na_i^+ accumulation that favors cardiac Na^+/Ca^{++} exchanger-mediated elevations in Ca_i^{++} and enhanced myocardial contractility [33]. Moreover, it was suggested that ouabain may also induce late I_{Na} through voltage-gated Na^+ channel phosphorylation by Ca^{++}/calmodulin-dependent kinase II (CaMKII) [34]. Ouabain was used here to increase Na_i^+ and thereby enhance cardiac Na^+/Ca^{++} exchanger-mediated Ca_i^{++} accumulation. Our present results show that ouabain prevented the inexcitability of rat ventricular tissue induced by R 50 ppm superfusion. This effect could be explained by R decrease of the Na_i^+ and an induced partial inhibition of cardiac Na^+/Ca^{++} exchanger with a decrease of Ca_i^{++}. Based on the second hypothesis we have tested the effect of BAY K 8644 [22], an L-type calcium channel agonist, when it was administered in the presence of R, our data show that, in the same extent of ouabain, that BAY K 8644 prevented

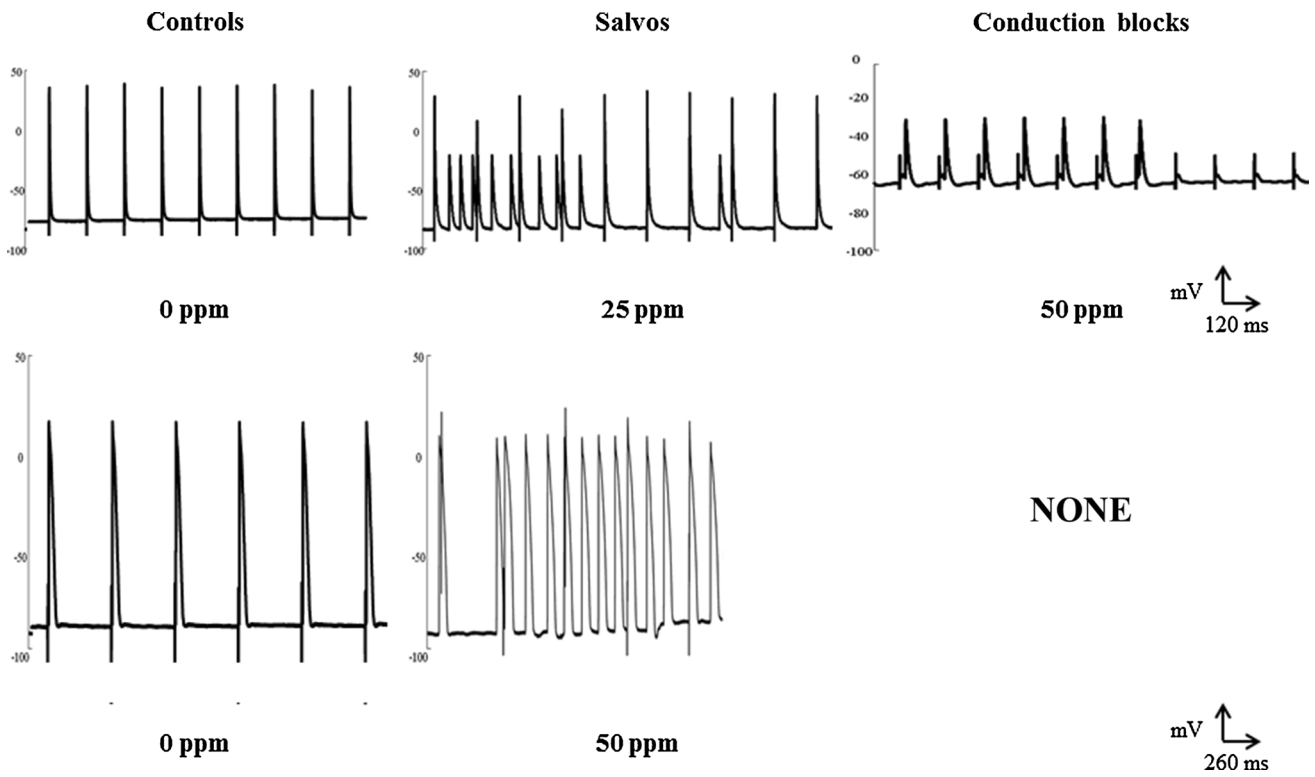


Fig. 5 Representative examples of serious arrhythmias (salvos) and conduction blocks occurring in ventricular tissues after Roundup. Rat (*upper panels*) and rabbit (*lower panels*) examples are illustrated. Under each section, ppm are indicated and 0 ppm represent controls. Note that in rabbit ventricular tissues, there was no conduction block (see also Table 1)

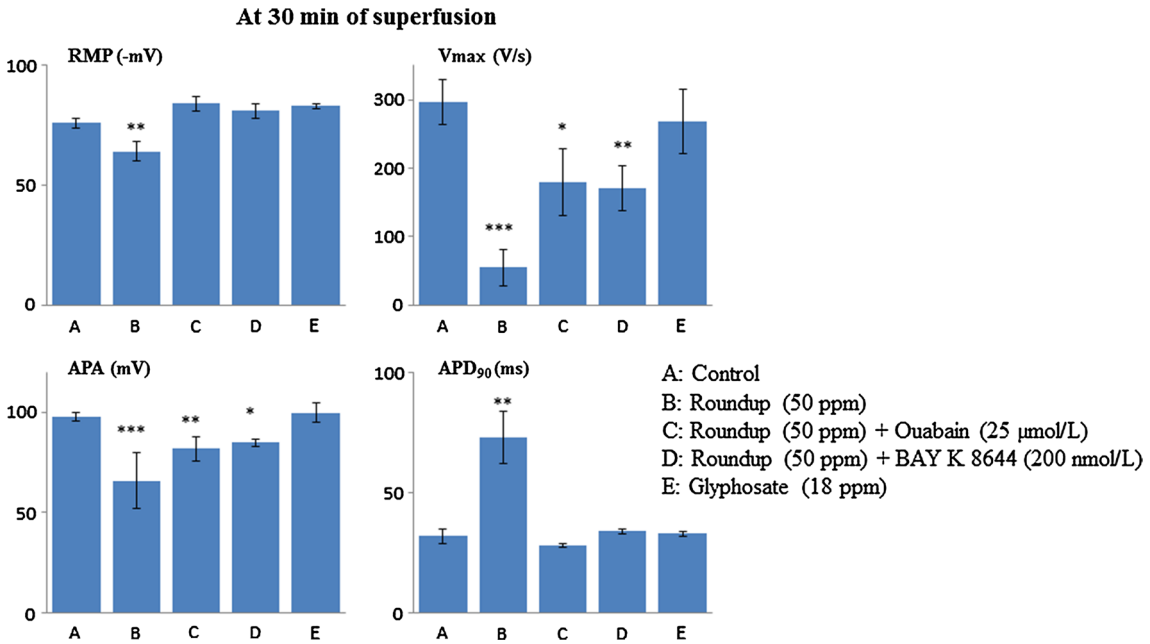


Fig. 6 Pharmacological interactions with Roundup 50 ppm at 30 min of superfusion. Both indirect (ouabain) and direct (BAY K 8644) interference with I_{Ca} induced less depolarization, less V_{max} decrease, less APA decrease and lower APD_{90} increase, pointing to a mechanistic role of I_{Ca} in the Roundup myocardial toxic effects (see text for details)

the inexcitability induced by R perfusion. It was previously demonstrated that the BAY K 8644 induced direct stimulation of I_{Ca} [35]. We can then suppose that R decreased Ca_i^{++} directly via the inhibition of I_{Ca} or indirectly via the decrease of Na_i^+. The V_{max} reduction in rat ventricular myocardium is in accordance with a reduction of Na_i^+ [36]

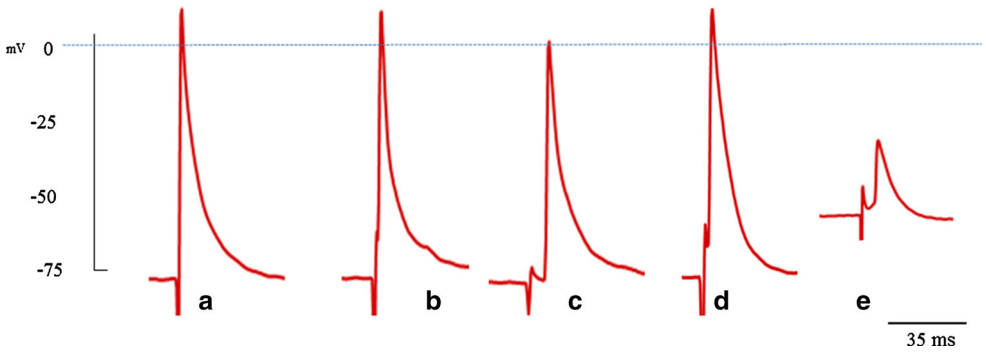


Fig. 7 Representative examples of rat right ventricular myocardial action potentials and the pharmacological interactions with Roundup 50 ppm at the end of 45 min superfusion: *a* = control, *b* = Glyphosate alone at 18 ppm, *c* = Roundup 50 ppm + ouabain 25 µmol/l, *d* = Roundup 50 ppm + BAY K 8644 200 nmol/l, *e* = Roundup alone at 50 ppm. Note that in *c* and *d* minimal, electrophysiological changes were observed as compared to *e*, whereas *b* was not different from *a*, pointing to the reduced effects of Roundup in the presence of both indirect (ouabain) and direct (BAY K 8644) interference with I_{Ca}

and of inward-going Na^+ current in the concentration range explored. Outward-going current which have slow (I_{Ks}), rapid (I_{Kr}) and ultrarapid (I_{Kur}) components [37] might be implicated in the lengthening of APD_{50} and APD_{90} in both rats and rabbits. However, from the present study, it is not possible to define which is the actual outward K^+ current inhibited by R (36 % of G) superfusion; thus, further studies are needed as concerns K^+ currents.

Our rat ventricular myocardial APs showed fast AP responses to stimulation, and R seemed to affect largely the Na^+-dependent phase 0 of the AP (Figs. 4, 5; Table 2). Where the resting potential had decreased sufficiently, at higher R concentrations, the AP took on the appearance of a Ca^{++}-dependent slow-response fiber. Moreover, a statistically significant decrease in dV/dt was measured for V_{max} (Table 2), also lower but not significantly so in rabbit ventricular myocardium (Table 3), which overall corroborate the probable Na^+-channel interference in ventricular muscle after R. Therefore, a further electrophysiological action that should be considered among the other discussed here clearly calls for specifically oriented investigations to be performed in the future, in order to confirm Na^+-current interference of R by single-channel studies.

Conduction blocks not recorded in control rat experiments were increased by R dose-dependently up to 500 ppm, whereas at higher doses there was complete inexcitability. This observation may be the myocardial tissue counterpart of the cellular inhibition of inward-going Na^+-current (i.e., V_{max} reduction). On the other hand, there was a significant arrhythmogenic effect of R in both rat and rabbit ventricular tissues as regards severe arrhythmias like salvos and tachycardia [24, 25]. Since the arrhythmogenic effects were accompanied by APD_{50} and APD_{90} prolongations, it is probable that these effects are of a toxic nature. In view of these proarrhythmic and conduction blocking effects, just the opposite of what is expected for a safe drug from the cardiovascular point of view [38, 39], it is hard to accept that G might be proposed as an anticancer agent [40] before deeper investigations are performed: In vitro studies of human atrial or ventricular tissues might be the next step.

Limitations

First, the study was performed in rat and rabbit myocardium, which although different from human myocardium present, especially in case of rabbits, main ionic currents qualitatively similar [24]: further in vitro studies of human tissues are highly recommended. Second, we did not use left ventricular myocardium because its excessive thickness was not compatible with our experimental model due to the risk of poor perfusion in the center of the tissue that could cause ischemia. Third, we were unable, for ethical reasons, to use in rabbit tissues the full range of doses used in rat tissues and accordingly no dose-relation was obtained in the former species. Overall, however, the evidence presented shows a dose-related tendency and this opens to future investigations to be performed in vivo. Fourth, ouabain and BAY K 8644 were investigated only in rat ventricular tissues. Finally, Ca_i^{++} was not measured directly which points to the need of further studies to be undertaken to confirm our electrophysiological conclusions.

Conclusions

In contrast to the general belief that R may be considered an inert component when animals or humans acutely ingest large quantities, the present electrophysiological study in

in vitro rat and rabbit heart ventricular tissues showed V_{max} and APD_{50} and APD_{90} changes after short superfusion and a high incidence of severe arrhythmias and of conduction blocks at the highest concentrations. Moreover, a 30-min washout with normal Tyrode solution did not enable recovery of normal electrophysiological parameters. These arrhythmias and conduction blocks may be explained by the effects of R on action potential parameters; indeed, the V_{max} decrease and APD_{50} and APD_{90} lengthening promote the occurrence of arrhythmias [35] and might represent significant I_{Na} and I_K blocking properties [36, 38], possibly related to the cation chelator ability [31] of G and to decreased $^{45}Ca^{++}$ influx through L-type voltage-dependent Ca^{++}-channels shown by both R and G [20] that our pharmacological studies support. Although further investigations are needed to also assess the exact nature of the K^+ current involved and Ca_i^{++} changes and to scan a large range of adjuvants [2], the evidence provided here strongly suggests that in rat and rabbit ventricular myocardium there are electrophysiological changes, conduction blocks and arrhythmias among GBH-mediated effects linking animal and human risks [12] after acute intoxications. In view of the scanty reports in the literature and a high probability of GBH toxic effects inducing death as the consequence of increased arrhythmogeneity, the results of the present study, the first ones assessing in vitro heart electrophysiology in two mammal species, should incite future in depth investigations aimed at detecting human risks [41].

Acknowledgments We wish to thank Josefa de Flores Martos Borrego to her technical help for these experiments. We thank also Nature Vivante and CRIIGEN for fellowship grants to Steeve Gress during the development of his Doctoral Thesis to obtain PhD degree in Molecular and Cellular Biology of the University of Caen Basse-Normandie, France.

Conflict of interest The authors declare that there are no conflicts of interest.

References

1. Mathur, P. P., & D'Cruz, S. C. (2011). The effect of environmental contaminants on testicular function. *Asian Journal of Andrology, 13*(4), 585–591. doi:10.1038/aja.2011.40.
2. Mesnage, R., Bernay, B., & Séralini, G.-E. (2013). Ethoxylated adjuvants of glyphosate-based herbicides are active principles of human cell toxicity. *Toxicology, 313*(2–3), 122–128. doi:10.1016/j.tox.2012.09.006.
3. Cavaş, T., & Könen, S. (2007). Detection of cytogenetic and DNA damage in peripheral erythrocytes of goldfish (Carassius auratus) exposed to a glyphosate formulation using the micronucleus test and the comet assay. *Mutagenesis, 22*(4), 263–268. doi:10.1093/mutage/gem012.
4. Cox, C., & Surgan, M. (2006). Unidentified inert ingredients in pesticides: Implications for human and environmental health. *Environmental Health Perspectives, 114*(12), 1803–1806.
5. Monosson, E. (2005). Chemical mixtures: Considering the evolution of toxicology and chemical assessment. *Environmental Health Perspectives, 113*(4), 383–390.
6. Séralini, G.-E., Clair, E., Mesnage, R., Gress, S., Defarge, N., Malatesta, M., & de Vendômois, J. S. (2014). Republished study: Long-term toxicity of a Roundup herbicide and a Roundup-tolerant genetically modified maize. *Environmental Sciences Europe, 26*(1), 14. doi:10.1186/s12302-014-0014-5.
7. Cassault-Meyer, E., Gress, S., Séralini, G.-É., & Galleraud-Denis, I. (2014). An acute exposure to glyphosate-based herbicide alters aromatase levels in testis and sperm nuclear quality. *Environmental Toxicology and Pharmacology*, *38*, 131–140. doi:10.1016/j.etap.2014.05.007.
8. Jeyaratnam, J. (1985). Health problems of pesticide usage in the Third World. *British Journal of Industrial Medicine, 42*(8), 505–506.
9. Jeyaratnam, J. (1985). 1984 and occupational health in developing countries. *Scandinavian Journal of Work, Environment & Health, 11*(3 Spec No), 229–234.
10. Kim, J., Ko, Y., & Lee, W. J. (2013). Depressive symptoms and severity of acute occupational pesticide poisoning among male farmers. *Occupational and Environmental Medicine, 70*(5), 303–309. doi:10.1136/oemed-2012-101005.
11. Kim, J., Shin, D.-H., & Lee, W. J. (2014). Suicidal ideation and occupational pesticide exposure among male farmers. *Environmental Research, 128*, 52–56. doi:10.1016/j.envres.2013.10.007.
12. Kim, Y. H., Lee, J. H., Hong, C. K., Cho, K. W., Park, Y. H., Kim, Y. W., & Hwang, S. Y. (2013). Heart rate-corrected QT interval predicts mortality in glyphosate-surfactant herbicide-poisoned patients. *The American Journal of Emergency Medicine,*. doi:10.1016/j.ajem.2013.09.025.
13. Lee, H. L., Chen, K. W., Chi, C. H., Huang, J. J., & Tsai, L. M. (2000). Clinical presentations and prognostic factors of a glyphosate-surfactant herbicide intoxication: A review of 131 cases. *Academic Emergency Medicine, 7*(8), 906–910.
14. Zouaoui, K., Dulaurent, S., Gaulier, J. M., Moesch, C., & Lachâtre, G. (2013). Determination of glyphosate and AMPA in blood and urine from humans: About 13 cases of acute intoxication. *Forensic Science International, 226*(1–3), e20–e25. doi:10.1016/j.forsciint.2012.12.010.
15. Jeyaratnam, J. (1990). Acute pesticide poisoning: A major global health problem. *World Health Statistics Quarterly. Rapport Trimestriel de Statistiques Sanitaires Mondiales, 43*(3), 139–144.
16. Chan, Y.-C., Chang, S.-C., Hsuan, S.-L., Chien, M.-S., Lee, W.-C., Kang, J.-J., & Liao, J.-W. (2007). Cardiovascular effects of herbicides and formulated adjuvants on isolated rat aorta and heart. *Toxicology In Vitro, 21*(4), 595–603. doi:10.1016/j.tiv.2006.12.007.
17. Koyama, K., Koyama, K., & Goto, K. (1997). Cardiovascular effects of a herbicide containing glufosinate and a surfactant: In vitro and in vivo analyses in rats. *Toxicology and Applied Pharmacology, 145*(2), 409–414. doi:10.1006/taap.1997.8196.
18. Testud, F., Grillet, J.-P., & Testud, F. (2007). *Produits phytosanitaires: Intoxications aiguës et risques professionnels*. Paris: Éditions Eska.
19. Schiariti, M., Puddu, P. E., & Rouet, R. (1994). Multivariate prediction of spontaneous repetitive responses in ventricular myocardium exposed in vitro to simulated ischemic conditions. *International Journal of Cardiology, 45*(1), 9–22.
20. De Liz Oliveira Cavalli, V. L., Cattani, D., Heinz Rieg, C. E., Pierozan, P., Zanatta, L., Benedetti Parisotto, E., & Zamoner, A. (2013). Roundup disrupts male reproductive functions by triggering calcium-mediated cell death in rat testis and Sertoli cells. *Free Radical Biology and Medicine, 65*, 335–346. doi:10.1016/j.freeradbiomed.2013.06.043.

21. Pasdois, P., Quinlan, C. L., Rissa, A., Tariosse, L., Vinassa, B., Costa, A. D. T., & Garlid, K. D. (2007). Ouabain protects rat hearts against ischemia-reperfusion injury via pathway involving src kinase, mitoKATP, and ROS. *American Journal of Physiology Heart and Circulatory Physiology, 292*(3), H1470–H1478. doi:10.1152/ajpheart.00877.2006.
22. Bechem, M., & Hoffmann, H. (1993). The molecular mode of action of the Ca agonist (−) BAY K 8644 on the cardiac Ca channel. *Pflügers Archiv: European Journal of Physiology, 424*(3–4), 343–353.
23. King-Herbert, A. P., Sills, R. C., & Bucher, J. R. (2010). Commentary: Update on animal models for NTP studies. *Toxicologic Pathology, 38*(1), 180–181. doi:10.1177/0192623309356450.
24. Puddu, P. E., Legrand, J.-C., Sallé, L., Rouet, R., & Ducroq, J. (2011). I(Kr) vs. I(Ks) blockade and arrhythmogenicity in normoxic rabbit Purkinje fibers: Does it really make a difference? *Fundamental & Clinical Pharmacology, 25*(3), 304–312. doi:10.1111/j.1472-8206.2010.00920.x.
25. Rouet, R., Picard, S., Criniti, A., Monti, F., Dawodu, A. A., Ruvolo, G., & Puddu, P. E. (1999). Effects of bimakalim on human cardiac action potentials: Comparison with guinea pig and nicorandil and use-dependent study. *Journal of Cardiovascular Pharmacology, 33*(2), 255–263.
26. Cheng, J. (2006). Evidences of the gender-related differences in cardiac repolarization and the underlying mechanisms in different animal species and human. *Fundamental & Clinical Pharmacology, 20*(1), 1–8. doi:10.1111/j.1472-8206.2005.00384.x.
27. Spear, J. F., & Moore, E. N. (2000). Gender and seasonally related differences in myocardial recovery and susceptibility to sotalol-induced arrhythmias in isolated rabbit hearts. *Journal of Cardiovascular Electrophysiology, 11*(8), 880–887.
28. Zahradníková, A., Minarovic, I., & Zahradník, I. (2007). Competitive and cooperative effects of Bay K8644 on the L-type calcium channel current inhibition by calcium channel antagonists. *The Journal of Pharmacology and Experimental Therapeutics, 322*(2), 638–645. doi:10.1124/jpet.107.122176.
29. Séralini, G.-E., Mesnage, R., Defarge, N., Gress, S., Hennequin, D., Clair, E., & de Vendômois, J. S. (2013). Answers to critics: Why there is a long term toxicity due to a Roundup-tolerant genetically modified maize and to a Roundup herbicide. *Food and Chemical Toxicology, 53*, 476–483. doi:10.1016/j.fct.2012.11.007.
30. Yao, J. A., & Tseng, G. N. (1997). Azimilide (NE-10064) can prolong or shorten the action potential duration in canine ventricular myocytes: Dependence on blockade of K, Ca, and Na channels. *Journal of Cardiovascular Electrophysiology, 8*(2), 184–198.
31. Olorunsogo, O. O. (1990). Modification of the transport of protons and Ca^{2+} ions across mitochondrial coupling membrane by *N*-(phosphonomethyl)glycine. *Toxicology, 61*(2), 205–209.
32. Schoner, W., & Scheiner-Bobis, G. (2007). Endogenous and exogenous cardiac glycosides and their mechanisms of action. *American Journal of Cardiovascular Drugs: Drugs, Devices, and Other Interventions, 7*(3), 173–189.
33. Saini, H. K., & Dhalla, N. S. (2007). Sarcolemmal cation channels and exchangers modify the increase in intracellular calcium in cardiomyocytes on inhibiting Na^{+}-K^{+}-ATPase. *American Journal of Physiology Heart and Circulatory Physiology, 293*(1), H169–H181. doi:10.1152/ajpheart.00007.2007.
34. Hoyer, K., Song, Y., Wang, D., Phan, D., Balschi, J., Ingwall, J. S., & Shryock, J. C. (2011). Reducing the late sodium current improves cardiac function during sodium pump inhibition by ouabain. *The Journal of Pharmacology and Experimental Therapeutics, 337*(2), 513–523. doi:10.1124/jpet.110.176776.
35. Rohr, S., & Kucera, J. P. (1997). Involvement of the calcium inward current in cardiac impulse propagation: Induction of unidirectional conduction block by nifedipine and reversal by Bay K 8644. *Biophysical Journal, 72*(2 Pt 1), 754–766.
36. Hondeghem, L. M. (1978). Validity of V_{max} as a measure of the sodium current in cardiac and nervous tissues. *Biophysical Journal, 23*(1), 147–152. doi:10.1016/S0006-3495(78)85439-3.
37. Rudy, Y. (2008). Molecular basis of cardiac action potential repolarization. *Annals of the New York Academy of Sciences, 1123*, 113–118. doi:10.1196/annals.1420.013.
38. Jouve, R., Langlet, F., Puddu, P. E., Rolland, P. H., Guillen, J. C., Cano, J. P., & Serradimigni, A. (1986). Cicletanide improves outcome after left circumflex coronary artery occlusion-reperfusion in the dog. *Journal of Cardiovascular Pharmacology, 8*(1), 208–215.
39. Picard, S., Rouet, R., Ducouret, P., Puddu, P. E., Flais, F., Criniti, A., & Gérard, J. L. (1999). KATP channels and «border zone» arrhythmias: Role of the repolarization dispersion between normal and ischaemic ventricular regions. *British Journal of Pharmacology, 127*(7), 1687–1695. doi:10.1038/sj.bjp.0702704.
40. Li, Q., Lambrechts, M. J., Zhang, Q., Liu, S., Ge, D., Yin, R., & You, Z. (2013). Glyphosate and AMPA inhibit cancer cell growth through inhibiting intracellular glycine synthesis. *Drug Design, Development and Therapy, 7*, 635–643. doi:10.2147/DDDT.S49197.
41. Gress, S., Lemoine, S., Séralini, G.-E., & Puddu, P.E. (2014). Glyphosate-based herbicides potently affect cardiovascular system in mammals: Review of the literature. *Cardiovascular Toxicology*. doi: 10.1007/s12012-014-9282-y.