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Mortal Furosemide-Hypokalemia-Disturbances in Rats NO-System Related Shorten Survival by L-NAME. Therapy Benefit with BPC 157 Peptide More Than With L-Arginine

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Abstract

Background: We focused on NO-system-relations (worsening/amelioration) of furosemide (100 mg/kg intraperitoneally)-diuresis-hypokalemia mortal course in rats and beneficial effect of BPC 157 therapy.

Methods: Electrocardiographically 90-150 min post-furosemide application duration of PR, RR, QRS, QT intervals, P, R, S, T waves and its amplitude as well were analysed along with appearance of AV block, ventricular premature beats, ventricular tachycardia. Clinically, skeletal muscle myoclonal activity and lethality at 150 min were also analysed.

Results: All NO-system-related agents (alone and/or combined, before/after furosemide) not changed hypokalemia and all averted to some extent furosemide-forced diuresis. NOS-blocker, L-NAME (5 mg/kg intraperitoneally) accelerated mortality, aggravated cardiac and extra-cardiac manifestations, thereby, NO-system-related. Prevented hypokalemia-mortality was with NO-precursor L-arginine (100 mg/kg intraperitoneally) and stable gastric pentadecapeptide BPC 157 (10 ug, 10 ng/kg intraperitoneally/intragastrically). Specifically, BPC 157 showed most complete benefit. i. BPC 157 given 15 min before furosemide. All BPC 157 regimens maintained sinus rhythm, had no ventricular premature beats, ventricular tachycardia, AV block, no prolongation of intervals and waves without reduction of amplitude. ii. BPC 157 given 90 min after furosemide (with hypokalemia, 3rd grade AV block and/or ventricular tachycardia being present). Within 5-10 minutes, BPC 157 regimens normalized P, R, S, T waves, PR, RR, QRS, QT interval duration, R, S, T wave amplitude, total AV block and terminated ventricular tachycardia. Likewise, BPC 157 eliminated skeletal muscle myoclonus.

Conclusion: L-NAME/L-arginine was mutual counteraction while BPC 157 completely eliminated L-NAME (arrhythmias, myoclonus, mortality), without an additive benefit when combined with L-arginine. Thus, we showed potentially effective therapeutic interventions for acute hypokalemia.

Keywords: Pentadecapeptide BPC 157; L-NAME; L-arginine; Hypokalemic lethal outcome; Arrhythmia; Rats

Introduction

NO-system is proposed as endogenous cardioprotectant and antifibrillatory factor [1,2]. Diuretic-hypokalemia is an increasingly common cause of arrhythmias [3]. Therefore, we attempted to define hypokalemia and/or hypokalemia disturbances as particular disturbances related to NO-system and possible therapeutic value of NO-system-related agents: NO-synthase (NOS)-blocker, L-NAME, NOS-substrate, L-arginine, and, particularly, stable gastric pentadecapeptide BPC 157 [4-6]. In this, we focused on so far not demonstrated with L-NAME/L-arginine application [1,2] NO-system-relations in mortal furosemide-diuresis-hypokalemia course; -ECG changes, carefully analyzed in rats [7] (prolongation of P, R, S, T waves and PR, RR, QRS, QT interval duration, and reduced amplitude of R, S, T wave (although not completely related to hypokalemia) [7]); -arrhythmias (AV conduction block, ventricular premature beats, ventricular tachycardia); -skeletal muscle myoclonus [8]; and -lethality 90-150 min post-furosemide. Likely, this furosemide-hypokalemia-NO-system-syndrome should be worsened by L-NAME, ameliorated by L-arginine and finally, counteracted by BPC 157. Namely, BPC 157 interacts with NO-systems in prevention of chronic heart failure, reversal of already established chronic heart failure [9], preventing digitalis arrhythmias and counteracting already advanced digitalis arrhythmias [10], both in vivo and in vitro, always overriding the L-arginine's cardioprotectant and antiarrhythmic effect [10-14]. Since the methyldigoxin toxicity depends particularly on hypokalemia [1,2,15], an analogous life saving effect of BPC 157 would occur in hypokalemic rats overloaded with a huge dose of furosemide. Of note, probably also important for expected cardioprotectant and antiarrhythmic effectiveness [9,10], BPC 157 (particularly effective in the whole gastrointestinal tract (GEPPPGKPADDAGLV, M.W. 1419, in trials for inflammatory bowel disease), wound treatment [4-6], stable and not degraded in human gastric juice (more than 24h)) represents a well-matched anti-ulcer

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peptide [4-6]. Producing no reported toxicity, it is effective alone without carrier [4-6], positively targeting particularly vasculature (i.e., removal of vascular clot) [16] and recently, reducing bleeding time and thrombocytopenia after heparin, warfarin or aspirin [17]. Thus, using furosemide over-load without potassium intravenous supplementation in rats as a background in present study (life treating hypokalemia (<2.7 mEq/L)), BPC 157 was applied intraperitoneally or intragastrically, alone or in combination with L-NAME and/or L-arginine. Medication was given prophylactically before furosemide or alternatively, as a therapy, after furosemide, when hypokalemia-third-degree AV block and ventricular tachycardia were already established. To prove possible general mechanism how BPC-157 could protect cells in hypokaliemic conditions we used HEK293 cell. Membrane voltages (V_m) of HEK293 cells were measured using the slow-whole-cell patch clamp technique [18].

Material and Methods

Animals

Female Wistar Albino rats (190-220 g) randomly assigned (10 rats per each experimental group), were used (Medical Faculty, University of Zagreb). All experiments approved by the Local Ethics Committee.

Medication, ECG recording and assessment

The furosemide (Furosemid, Pliva) dose (100 mg/kg i.p.) that was used was considerably increased [7] to demonstrate that after such forced diuresis, there is an unfailing acute hypokalemic syndrome (serum potassium level (<3 mmol/L); ECG changes (prolonged P, R, S, T waves and PR, RR, QRS, QT interval duration along with reduced amplitude of R, S, T wave although not completely related to hypokalemia [7]; arrhythmias (AV block, ventricular premature beats, ventricular tachycardia) and skeletal muscles myoclonus presentation)) that would be invariably lethal without curative agent application. Pentadecapeptide BPC 157 (manufactured by Diagen, Ljubljana, Slovenia [4-6,9,10], (GEPPPGKPADDAGLV, M.W. 1419, partial sequence of human gastric juice protein BPC, peptide with 99% purity (HPLC, freely soluble in water at pH 7.0 and in saline) dissolved in saline or saline alone (5.0 ml/kg), were intraperitoneally or intragastrically given 15 minutes before the intraperitoneal injection of 100 mg/kg of furosemide (Edemid forte, Belupo) (prophylactic regimen). BPC 157 rats that received intraperitoneal or intragastrical application, received the dose of the 10 μg/kg b.w. or the dose of the 10 ng/kg b.w.. In addition, L-NAME (5 m/kg) and/or L-arginine (100 mg/kg) (and D-arginine (Sigma, St. Louis, MO, USA) were dissolved in saline as described before [10-14]) were given intraperitoneally. Alternatively, the described medication was given intraperitoneally or intragastrically at 90 minutes after furosemide (therapeutic regimen). In combination studies, L-NAME and/or L-arginine (since without effect, D-arginine was not further shown) were given with BPC 157 10 μg/kg intraperitoneally (L-NAME+BPC 157; L-arginine+BPC 157; L-NAME+L-arginine+BPC 157).

Generally, through the 90 min period after furosemide application, the rats were monitored (separate clinical assessment and urination (mL) in metabolic cage for rat (Ugo Basile) at 5, 15, 30, 45, 60, 75 and 90 min after furosemide).

At 90 min after furosemide, the rats were deeply anesthetized (Thiopental-Natrium Pulver 40 mg/kg i.p. plus diazepam 5 mg/kg i.p.), and the ECG was recorded continuously throughout the next 60 minutes in three main leads by positioning stainless steel electrodes on all four limbs, using an ECG monitor by Medtronic Analyser programmer (Minneapolis, MN, USA). The ECG assessment included analysis of wave (P, R, S, T) amplitude and duration, interval (PQ, QRS,RR, QT) duration, time (s) until the start of third-degree AV block, its duration (s), time (min) until onset of ventricular tachycardia and the duration (min) of ventricular tachycardia. Ventricular tachycardia was defined as a run of three or more premature QRS complexes, not to be defined in terms of its rate or the prevailing sinus rate [19]. Total atrioventricular block was defined when no supraventricular impulses conducted to the ventricles and consequently in electrocardiografphical finding P waves which reflect a sinus node rhythm were independent from QRS complexes.

In the studies of the agents effect on already established severe hypokalemia disturbances, 90 min after the furosemide challenge, significant hypokalemia was recognized by ECG signs (reduction of R, S, T wave amplitude, prolongation of P, R, S, T wave duration as well as PQ, RR, QT interval duration along with third-degree AV block and/or ventricular tachycardia appearance) and ECG changes were monitored until the end of the next 60 min period (which however, could not be further extended because of the fatal outcome, regularly occurring at that time).

In a separate group of rats, blood samples were collected using a heparin tube from the rat eye before furosemide challenge, and thereafter at the time of recognized hypokalemia by ECG signs at 90 min and at the end of 60 min-observation period (i.e., 150 min after furosemide challenge). The concentrations of plasma electrolytes were measured by an autoanalyzer (Olympus AU2700 analyzer).

The duration of myoclonus was accordingly assessed. Myoclonus is defined as sudden, brief, shock-like, involuntary movements caused by muscular contraction or inhibitors [20].

The time until death and the number of fatal outcomes (assessed by ECG, asystolia and lack of breathing for more than 3 minutes) in hypokalemia-induced arrhythmias was also assessed.

Cell culture

HEK293 cells were grown on glass coverslips in Dulbecco's Modified Eagle Medium (DMEM) which contained 3.7 g/l $NaHCO_3$ in addition of 2 mM L-glutamine, 10 ml/l Penicilin/Streptomicin (10000E/10000 mg/ml) and 10% fetal calf serum (FCS). Cells were kept at 5% CO_2 at 37°C and used from experiments at passage 190, 5-6 days after trypsination (0.25% trypsin-EDTA solution, Sigma-Aldrich in Mg^{2+}, Ca^{2+} free HANKS solution).

Patch clamp studies

Coverslips with HEK293 cells were mounted as the bottom of a perfusion chamber on an inverted microscope (Axiovert 10 Zeiss, Gottingen, Germany). Membrane voltages (V_m) of HEK293 cells were measured using the slow-whole-cell patch clamp technique [18]. A solution containing 145 mM NaCl, 1.6 mM K_2HPO_4, 0.4 mM KH_2PO_4, 5 mM D-glucose, 1 mM $MgCl_2$, 1.3 mM calcium gluconate (pH 7.4) was used in experiments. All experiments were performed at 37°C with a bath perfusion rate of 10 ml/min. Patch clamp pipettes were filled with 95 mM potassium gluconate, 30 mM KCl, 4.8 mM Na_2HPO_4, 1.2 mM NaH_2PO_4, 5 mM D-glucose, 1.3 mM calcium gluconate, 1.03 mM $MgCl_2$, and 1 mM ATP (pH 7.2). To this solution 160 μM nystatin were added to permeabilize the membrane under the pipette. Pipette resistance prepared this way had resistance of 5-10 MΩ V_m was measured with a patch-clamp amplifier (U. Frobe, Physiologische Institut, Freiburg,

Germany) and recorded continuously on a pen recorder (WeKa graph, Kaltbrunn, Switzerland). All effects were analysed after V_m had reached a new steady state value and were compared to their averaged pre- and post control values.

To determine effects of BPC-157 on V_m of HEK293 cells during hypokalemic condition (hypokaliemic step), we used following solution: 145 mM NaCl, 1.6 mM Na_2HPO_4, 0.4 mM KH_2PO_4, 5 mM D-glucose, 1 mM $MgCl_2$, 1.3 mM calcium gluconate (pH 7.4). This solution has potassium concentration of 0.4 mM which is hypokalemic compared to control solution ($[K^+]$ = 3.6 mM). After first hypokaliemic step (pre-control), cells were overperfused with BPC-157 (1 μM) for at least 2 min. Hyperkalemic step was repeated together with BPC-157 in the bath solution. After washing BPC-157 for few minutes, additional hypokalemic step was performed (post-control).

Statistical analysis

Statistical analysis was performed using parametric two-way mixed model ANOVA (one factor is repeated-measures) and Student Newman–Keuls test to compare the difference between groups. Fisher's exact probability test was used to assess the number of dead and surviving rats. A P value of 0.05 or less was considered statistically significant.

Results

Plasma potassium and other electrolyte concentrations; furosemide induced diuresis

Always, after furosemide overdose, steady severe hypokalemia (<2.7 mmol/L) (Tables 1 A and 1B) is equally persisting, even after all agents averted to some extent furosemide-forced diuresis. In particular, L-arginine, BPC 157 postponed furosemide-diuresis. L-NAME showed a decrease of furosemide-diuresis, antagonized by L-arginine or BPC 157 (Tables 2A and 2B).

Severe acute hypokalemia syndrome (control rats presentation)

All hypokalemic rats consistently exhibited prolongation of the PQ, QRS, QT (Tables 3A, 3B, 4A and 4B), RR (Tables 5A and 5B) interval and all wave (P, R, S, T) duration, with a concomitant decrease of the amplitude of all waves except the P wave (Tables 3A, 3B, 4A, 4B), considerable number of premature ventricular beats (per min), marked duration of ventricular tachycardia, quite extensive duration of 3°AV block (min) (Table 6), no termination of arrhythmias, lethality was absolute and fatal outcome invariably occurred at the end of the

Electrolytes plasma concentrations (mmol/L) course in rats before (A) and after (B) furosemide, with or without BPC 157 medication

A. Course in rats before furosemide					Drug protocol: Furosemide challenge	B. Course in rats after furosemide							
Route	Drug protocol: saline, BPC 157, Nothing	Electrolytes plasma concentrations (mmol/L)				Electrolytes plasma concentrations (mmol/L)			Drug protocol: nothing, saline, BPC 157	Electrolytes plasma concentrations (mmol/L)			Regimen
		K	Na	Cl		K	Na	Cl		K	Na	Cl	
i.p.	saline+	4.4±0.7	134.0±1.1	80.2±4.1	+Furosemide	2.70±0.24*	135.25±2.1	81.25±6.06	/	2.70±0.19*	134.00±2.5	79.9±4.7	PROPHYLACTIC
	BPC157 10μg/kg+	4.3±0.6	133.8±1.3	77.9±5.3	+Furosemide	2.87±0.23*	134.5±0.87	79.75±2.95	/	2.74±0.15*	134±0.71	81.9±5.0	
	BPC157 10ng/kg+	4.2±0.3	133.7±0.8	79.2±2.7	+Furosemide	2.87±0.26*	132.25±2.28	76.5±4.77	/	2.78±0.19*	132.7±1.09	76.2±2.6	
i.g.	saline+	4.7±0.6	136.7±1.1	80.2±4.1	+Furosemide	2.91±0.24*	130.55±2.1	81.25±6.06	/	2.80±0.39*	138.0±0.95	79.4±3.7	
	BPC157 10μg/kg+	4.1±0.8	137.8±1.5	78.9±7.3	+Furosemide	2.77±0.38*	132.7±0.67	77.15±3.97	/	2.64±0.15*	134.9±0.71	81.0±5.0	
	BPC157 10ng/kg+	4.6±0.3	132.9±0.7	78.8±2.7	+Furosemide	2.67±0.18*	134.55±3.28	79.9±5.77	/	2.88±0.32*	138.7±2.1	77.2±5.6	
i.p.	/	*4.3±0.5*	*134.0±1.6*	*78.8±3.9*	Furosemide+	2.72±0.18*	135.0±1.58	79.8±4.15	+ saline	2.8±0.1*	135.0±1.92	80.2±3.3	THE APEUTIC R
	/	*4.1±0.8*	*133.9±1.8*	*80.5±2.9*	Furosemide+	2.86±0.23*	138.9±1.26	81.0±3.63	+ BPC157 10μg/kg	2.74±0.15*	134.8±0.95	81.5±4.9	
	/	*4.3±0.4*	*134.4±1.8*	*79.4±5.1*	Furosemide+	2.84±0.27*	132.9±2.07	76.6±4.77	+ BPC157 10ng/kg	2.76±0.13*	138.5±2.45	76.8±1.9	
i.g.	/	*4.8±0.6*	*134.9±2.7*	*80.8±4.9*	Furosemide+	2.72±0.18*	138.9±3.55	80.8±5.15	+ saline	2.6±0.1*	135.0±1.92	78.2±5.3	
	/	*4.3±0.8*	*135.7±3.8*	*79.5±3.9*	Furosemide+	2.86±0.23*	135.4±4.26	81.9±3.63	+ BPC157 10μg/kg	2.54±0.35*	134.8±0.71	81.9±6.0	
	/	*4.3±0.4*	*136.4±2.8*	*77.4±5.8*	Furosemide+	2.84±0.27*	134.9±3.05	78.6±5.77	+ BPC157 10ng/kg	2.46±0.33*	134.9±2.45	79.8±4.9	
	Medication at 15 min before furosemide	Presentation before furosemide challenge			Furosemide (100 mg/kg i.p.) challenge	Presentation at 90 minute after furosemide challenge			Medication at 90 min after furosemide	Presentation at 150 min after furosemide challenge			
										Presentation at 60 min after BPC 157/saline medication (therapeutic regimen)			

Table 1A: Electrolytes plasma concentrations (mmol/L), means ±SEM. BPC 157 administered intraperitoneally or intragastrically was given prophylactically before furosemide (100 mg/kg intraperitoneally) or alternatively, as a therapy, after furosemide, when hypokalemia- third-degree AV block and ventricular tachycardia were already established. * P<0.05, at least vs. *initial presentation (italic)* without furosemide.

Electrolytes plasma concentrations (mmol/L) course in rats before (A) and after (B) furosemide, with or without BPC 157 medication												
A. Course in rats before furosemide				Drug protocol: **Furosemide challenge**	B. Course in rats after furosemide							
Drug protocol: saline, BPC 157, BPC 157, L-NAME, L-arginine i.p. or nothing	Electrolytes plasma concentrations (mmol/L)				Electrolytes plasma concentrations (mmol/L)			Drug protocol: saline, BPC 157, BPC 157, L-NAME, L-arginine i.p. or nothing	Electrolytes plasma concentrations (mmol/L)			Regimen
	K	Na	Cl		K	Na	Cl		K	Na	Cl	
saline+	4.3±0.9	133.1±0.9	79.7±2.6	+Furosemide	2.7±0.3*	134.3±1.5	80.5±3.7	/	2.7±0.2*	134.2±25	79.4±2.7	PROPHYLACTIC
BPC157 10µg/kg+	4.4±0.3	133.7±1.1	78.5±3.2	+Furosemide	2.8±0.2*	134.5±1.1	79.7±3.1	/	2.7±0.4*	134.1±0.6	80.7±2.9	
L-NAME+	4.3±0.9	133.5±1.0	78.4±1.9	+Furosemide	2.9±0.3*	133.5±1.8	77.7±2.9	/	2.9±0.5*	133.7±1.1	78.8±1.9	
L-NAME + BPC 157	4.2±0.8	133.5±0.9	79.2±2.3	+Furosemide	2.7±0.2*	134.8±2.1	78.9±3.5	/	2.8±0.5*	133.7±1.5	80.6±2.1	
L-arginine+	4.3±0.4	134.1±1.8	79.3±3.1	+Furosemide	2.8±0.1*	134.2±1.6	80.1±2.6	/	2.7±0.2*	134.1±1.3	80.3±3.2	
L-arginine + BPC 157+	4.2±0.5	133.9±1.4	79.8±2.5	+Furosemide	2.7±0.3*	133.8±2.2	80.3±2.1	/	2.7±0.2*	135.0±1.7	79.2±2.5	
L-NAME + L-arginine+	4.3±0.4	134.1±1.8	79.3±3.1	+Furosemide	2.8±0.2*	135.1±0.9	79.6±2.5	/	2.8±0.1*	133.9±1.2	80.9±1.8	
L-NAME + L-arginine + BPC 157+	4.4±0.7	133.9±1.4	79.8±2.5	+Furosemide	2.7±0.3*	134.7±1.7	81.1±3.1	/	2.7±0.2*	134.2±2.1	81.2±2.2	C
/	*4.4±0.9*	*133.1±1.2*	*79.7±2.5*	Furosemide+	2.6±0.3*	133.3±1.6	81.5±3.4	+ saline	2.8±0.3*	134.5±1.8	78.9±2.8	THERAPEUTIC
/	*4.3±0.7*	*132.7±1.0*	*78.5±3.0*	Furosemide+	2.8±0.2*	133.5±1.8	78.7±3.1	+BPC 157	2.7±0.4*	134.2±1.9	80.1±1.9	
/	*4.3±0.7*	*134.5±1.3*	*79.4±2.0*	Furosemide+	2.8±0.2*	134.5±1.4	79.7±3.0	+L-NAME	2.9±0.2*	133.7±2.1	77.9±3.2	
/	*4.2±0.8*	*134.5±0.9*	*79.2±1.9*	Furosemide+	2.6±0.3*	133.8±2.0	77.9±3.1	+L-NAME + BPC 157	2.8±0.1*	135.1±1.5	81.0±2.4	
/	*4.3±0.5*	*134.1±1.6*	*78.3±3.2*	Furosemide+	2.7±0.2*	133.2±1.6	79.9±2.6	+ L-arginine	2.7±0.1*	134.4±2.3	79.5±2.1	
/	*4.3±0.5*	*134.9±1.5*	*79.8±2.1*	Furosemide+	2.8±0.3*	133.8±2.3	80.1±2.2	+ L-arginine + BPC 157	2.8±0.3*	133.4±1.9	77.8±2.6	
/	*4.3±0.7*	*132.1±1.4*	*79.3±3.0*	Furosemide+	2.8±0.3*	134.1±1.4	79.6±2.0	+L-NAME + L-arginine	2.8±0.4*	134.8±1.7	81.1±2.8	
/	*4.3±0.7*	*133.9±1.5*	*79.8±2.3*	Furosemide+	2.6±0.3*	134.7±1.8	80.4±2.9	+L-NAME + L-arginine + BPC 157	2.7±0.3*	134.1±1.5	80.5±3.1	R
Medication at 15 min before furosemide	Presentation before furosemide challenge			Furosemide (100 mg/kg i.p.) challenge	Presentation at 90 minute after furosemide challenge			Medication at 90 min after furosemide	Presentation at 150 min after furosemide challenge			
									Presentation at 60 min after BPC 157, L-NAME, L-arginine/saline medication (therapeutic regimen)			

Table 1B: Electrolytes plasma concentrations (mmol/L), means ±SEM. L-NAME and L-arginine administered intraperitoneally prophylactically before furosemide (100 mg/kg intraperitoneally) or alternatively, as a therapy, after furosemide, when hypokalemia- third-degree AV block and ventricular tachycardia were already established. *P<0.05, at least vs. *initial presentation (italic)* without furosemide.

observation period (Tables 6, 7A and 7B). In particular, along with the above mentioned ECG changes, furosemide rats also developed AV conduction block (including third degree) in Hiss-Purkinje system (3rd degree AV block with wide QRS complexes (duration: 30-33 msec vs normal range 15-17.5 msec) and low ventricular frequency (90-120/min vs. normal range 320-500/min) and polymorphic ventricular tachycardia, frequency between 270-290/min with also wide QRS complexes (duration 30-33 msec) and clockwise rotation of electrical axis (Table 8)) and an appearance of abnormal ventricular rhythm (multifocal ventricular premature beats or polymorphic ventricular tachycardia („torsades de pointes“) (Table 8). Along with hypokalemia-induced ECG abnormalities, all control rats manifest myoclonus i.e., sudden, brief, shock-like, involuntary movements throughout the entire 60 min test period (i.e., 90-150 minutes after furosemide) (Table 9).

Stable gastric pentadecapeptide BPC 157

All BPC 157-furosemide rats survived. In general, in all hypokalemic BPC 157 rats AV block and abnormal ventricular rhythm either did not appear (BPC 157 before furosemide) or in rats presenting with the

Medication at 15 min before furosemide	Cumulative amount of urine in a given time interval (ml)						
	Time after furosemide (post-furosemide time) (min)						
	5	15	30	45	60	75	90
	Time after medication application (post-therapy time) (min)						
	20	30	45	60	75	90	105
ONTROL (saline 5ml/kg i.p.)¥	0	0	1.1±0.9	3.2±1.4	5.0±1.5	6.2±1.8	7.6±1.8
BPC 10µg/kg i.p.¥	0	0	0*	0.8±0.7*	2.8±2.2*	5.9±2.2	7.7±1.9
BPC 10ng/kg i.p.¥	0	0	0*	0.3±0.2*	2.9±0.7*	5.5±0.7	7.5±1.7
CONTROL (saline 5ml/kg i.g.)¥	0	0	1.1±0.8	3.4±0.5	5.1±0.9	6.3±0.9	7.9±1.2
BPC 10µg/kg i.g.¥	0	0	0*	1.1±0.8*	3.1±1.5*	6.2±2.0	7.8±1.5
BPC 10ng/kg i.g.¥	0	0	0*	1.0±0.9*	3.3±1.3*	6.0±1.2	7.5±1.5

Table 2A: Effects of BPC 157 on furosemide (100 mg/kg intraperitoneally)-diuresis (mL). Values are mean ± SEM. ¥ - Two-way ANOVA with repeated measure on one factor (P<0.05) difference between measurements depends on group membership. *P<0.05 significantly different from control values.

Medication at 15 min before furosemide	Cumulative amount of urine in a given time interval (ml)						
	Time after furosemide (post-furosemide time) (min)						
	5	15	30	45	60	75	90
	Time after medication application (post-therapy time) (min)						
	20	30	45	60	75	90	105
CONTROL (SALINE 5ml/kg)¥	0	0	1.1±0.9	3.1±1.4	5.1±1.5	6.1±1.7	7.6±1.8
BPC 157¥	0	0	0*	0.9±0.8*	2.8±2.1*	5.9±2.2	7.6±1.9
L-NAME¥	0.2±0.1	0.3±0.1	0.5±0.1	0.8±0.3*	1.1±0.7*	2.1±0.6*	3.2±1.7*
L-NAME + BPC 157¥	0	0	0.4±0.8	1.7±0.4*	2.5±0.9*	3.7±0.8*	5.8±1.2*
L-arginine¥	0	0	0*	1.1±0.7*	3.1±1.4*	6.1±1.9	7.7±1.5
L-arginine + BPC 157¥	0	0	0*	1.2±0.9*	3.3±1.3*	5.9±1.1	7.5±1.4
L-NAME + L-arginine¥	0	0.2±0.1	0.9±*0.2	1.7±1.2*	2.2±1.3*	3.6±1.1*	4.6±1.8*
L-NAME + L-arginine + BPC 157¥	0	0	0*	1.2±0.9*	3.3±1.3*	5.9±1.1	7.2±1.2

Table 2B: Effects of BPC 157 (10 µg/kg i.p.), L-NAME (5 mg/kg i.p.) and L-arginine (100 mg/kg i.p.) on furosemide (100 mg/kg i.p.)-diuresis (mL). Values are mean ± SEM. ¥ - Two-way ANOVA with repeated measure on one factor (P<0.05) difference between measurements depends on group membership. *P<0.05 significantly different from control values.

third-degree AV block and ventricular tachycardia, sinus rhythm was completely restored within a few minutes after BPC 157. The myoclonus was either not exhibited (BPC 157 given before furosemide) or reversed within a very short period (BPC 157 given in rats with already advanced arrhythmias and myoclonus after furosemide) (minutes, means ± SEM) (i.e., 5.5 ± 0.5 (10 µg/kg i.p.), 9.5 ± 1.5 (10 ng/kg i.p.), 9.0 ± 1.5 (10 µg/kg i.g.), 11.0 ± 1.0 (10 ng/kg i.g.), (vs. controls P<0.05, at least)). Specifically, BPC 157 was fully effective intraperitoneally (Table 3A, Table 3B) or intragastrically (Tables 4A and 4B), alone (Table 3-6) or in combination with L-NAME and/or L-arginine (Tables 7A, 7B, 8, and 9), given prophylactically before furosemide (Tables 3A, 4A, 5A, 7A, 8 and 9) or alternatively, as a therapy, after furosemide (Tables 3B, 5B, 6B, 7B, 8 and 9), when hypokalemia-third-degree AV block and ventricular tachycardia were already established).

L-arginine

Given before or after furosemide as described, L-arginine attenuates arrhythmias and prevents a lethal outcome (Tables 7A, 7B and 8). L-arginine affected the extra-cardiac manifestations like it did the cardiac manifestations (Table 9).

L-NAME

Contrarily, given before or after furosemide, the effect was aggravation of arrhythmias and a more rapid lethal outcome (Tables 7A, 7B and 8). Also, L-NAME aggravated the extra-cardiac manifestations like it did the cardiac manifestations (Table 9).

Combinations (L-NAME+L-arginine; L-NAME+BPC 157; L-NAME+L-arginine +BPC 157; BPC 157+L-arginine)

Prophylactically (at health condition) as well as therapeutically (90 min after acute furosemide overdose) combining worsening (NO-synthase (NOS)-blocker, L-NAME) /amelioration (NOS-substrate, L-arginine) /nearly complete counteraction (BPC 157) lead to null mortality, cardiac/extra-cardiac manifestation rapidly eliminated (L-NAME+BPC 157; L-NAME+L-arginine+BPC 157 (thus, BPC 157 preserved beneficial effect, completely eliminated L-NAME harmful effect) ; L-arginine+BPC 157 (BPC 157 beneficial effect not potentiated)) (Tables 7A, 7B, 8 and 9). Alternatively, we noted mutual counteraction, mortality, cardiac/extra-cardiac manifestation like in controls (L-NAME+L-arginine) (Tables 7A, 7B, 8 and 9). Thereby, in summary, L-NAME>control>L-arginine>BPC 157, involves both early development, and late advanced hypokalemia-syndrome while hypokalemia was always the same.

Cell model

To prove possible general mechanism how BPC-157 could protect cells in hypokaliemic conditions we used HEK293 cell. Membrane voltages (V_m) of HEK293 cells were measured using the slow-whole-cell patch clamp technique [18]. In hypokalemic conditions (0.4 mM) cells hyperpolarized for -6.1 ± 1.1 mV, n=5 (Figure 1). After first hypokalemic step, we add to the solution 1 µM BPC-157 which depolarized cells for 4.6 ± 1.6 mV, n=5. Repeating hypokaliemic step in the presence of BPC-157, cells did not hyperpolarized (3.1 ± 1.6 mV, n=5, Figure 1). After washing BPC-157 from bath solution, under hypokalemic conditions cells hyperpolarized again (Figure 1).

Discussion

Possibly, these findings in severe acute hypokalemia syndrome in rats (life treating furosemide over-load without potassium intravenous

Before furosemide and before *intraperitoneal* medication		Medication at 15 min before furosemide	Time after furosemide (post-furosemide time) (min)						
			95	100	110	120	130	140	150
			Time after medication application (post-therapy time) (min)						
ECG amplitudes of P, R, S, T waves (0.01mV); P wave duration, QRS, QT, intervals duration (msec)			110	115	125	135	145	155	165
P wave amplitude (0.01mV)	2.6±0.8	CONTROL (saline 5ml/kg i.p.)	3.4± 0.8	3.3± 1.1	3.4± 0.5	3.4± 0.9	3.5± 1.1	3.4± 1.1	3.4± 1.3
	2.7±0.4	BPC 10µg/kg i.p.	2.5± 1.1	2.5± 0.8	2.5± 1.4	2.5± 1.5	2.5± 0.9	2.5± 1.2	2.5± 1.1
	2.5±0.3	BPC 10ng/kg i.p.	2.7± 0.6	2.7± 1.1	2.7± 0.5	2.7± 0.2	2.7± 1.1	2.7± 1.2	2.6± 1.1
R wave amplitude (0.01mV)	56±2.1	CONTROL (saline 5ml/kg i.p.)	39.2± 3.2	36.1± 2.7	36.0± 2.1	35.2± 3.2	35.1± 2.7	35.8± 2.2	35.7± 1.7
	58.9±1.3	BPC 10µg/kg i.p.	51.0± 2.2 †	51.5± 1.6 †	50.9± 1.3 †	50.1± 2.1 †	50.5± 1.6 †	51.1± 3.1 †	52.5± 1.6 †
	56.3±0.2	BPC 10ng/kg i.p.	55.2± 1.1 †	55.3± 1.2 †	54.3± 1.2 †	53.5± 2.1 †	53± 1.2 †	53± 1.9 †	53.2± 1.2 †
S wave amplitude (0.01mV)	21.4±1.8	CONTROL (saline 5ml/kg i.p.)	13.2± 2.4	12.9± 3.6	12± 1.8	12.2± 2.4	12.1± 3.6	12.4± 1.9	12.6± 2.6
	21.9±0.6	BPC 10µg/kg i.p.	22.6± 0.8 †	22.4± 1.5 †	22.9± 0.6 †	22.6± 0.8 †	23.4± 1.7 †	23.4± 0.8 †	23.1± 1.4 †
	22.3±1.1	BPC 10ng/kg i.p.	21.1± 1.1 †	23± 0.2 †	22.3± 1.1 †	21.1± 1.1 †	23± 0.6 †	22.2± 1.1 †	23.2± 1.6 †
T wave amplitude (0.01mV)	2.3±0.1	CONTROL (saline 5ml/kg i.p.)	1.5± 0.1	1.4± 1.3	1.4± 0.7	1.4± 0.3	1.4± 0.6	1.4± 0.6	1.4± 0.2
	2.1±0.3	BPC 10µg/kg i.p.	2.5± 1.1 †	2.5± 0.9 †	2.4± 1.1 †	2.5± 0.9 †	2.6± 1.1 †	2.5± 1.4 †	2.6± 1.3 †
	2.2±0.4	BPC 10ng/kg i.p.	2.1± 0.1 †	2.1± 0.9 †	2.1± 0.4 †	2.1± 0.9 †	2± 0.8 †	2.1± 1.1 †	2.2± 0.4 †
P wave duration (msec)	17±0.9	CONTROL (saline 5ml/kg i.p.)	25± 1.2	26.1± 2.2	27.2±1.4	27.8± 1.6	28.3± 1.6	28.5± 2.8	26.9± 1.2
	16±1.1	BPC 10µg/kg i.p.	17± 1.1 †	17.4± 0.9 †	17.9± 0.8 †	17.6± 0.7 †	17.9± 1.7 †	18± 2.1 †	18.1± 1.4 †
	17±1.3	BPC 10ng/kg i.p.	18± 1.3 †	17.1± 0.6 †	18± 1.4 †	19.1± 1.2 †	19.2± 1.5 †	19.3± 0.9 †	19.3± 1.4 †
QRS interval duration (msec)	16±0.3	CONTROL (saline 5ml/kg i.p.)¥	30± 1.1	31.8± 1.1	32.2± 1.1	32.7± 1.5	32.9± 2.2	33.2± 1.6	33.7± 1.4
	15±1.1	BPC 10µg/kg i.p.¥	17.1± 0.9 †	15.5± 1.7 †	15.5± 1.4 †	14.6± 0.7 †	14.5± 0.9 †	14.3± 1.4 †	14.1± 0.8 †
	16±0.8	BPC 10ng/kg i.p. ¥	20± 1.2 †	19.8± 1.3 †	17.5± 2.4 †	16.4± 0.7 †	16.1± 0.7 †	15.7± 0.9 †	14.9± 0.7 †
QT interval duration (msec)	82±1.2	CONTROL (saline 5ml/kg i.p.)¥	101± 1.1	103± 1.8	104.9± 0.7	105.7± 1.1	105.5± 0.9	105.6± 0.7	105.1± 0.5
	88±0.8	BPC 10µg/kg i.p.¥	75± 2.5 †	71.1± 2.4 †	70.8± 1.3 †	70.6± 0.9 †	70.3± 0.8 †	70.1± 1.2 †	70.2± 0.6 †
	85±1.5	BPC 10ng/kg i.p.¥	78± 3.1 †	73.1± 1.4 †	72.8± 1.8 †	71.8± 1.4 †	71.2± 0.9 †	71.1± 0.9 †	70.8± 1.4 †

Table 3A: Effects of hypokalemia on amplitudes (0.01mV) of ECG waves, ECG wave duration and interval duration (msec). BPC 157 administered intraperitoneally was given prophylactically before furosemide (100 mg/kg intraperitoneally). Values are mean ± SEM. ¥ - Two-way ANOVA with repeated measure on one factor ($P<0.05$) difference between measurements depends on group membership. †$P<0.05$ significantly different from control values.

supplementation, BPC 157, intraperitoneally or intragastrically, alone or in combination with L-NAME and/or L-arginine) might contribute to understanding of K+ homeostatis and on the myocardial conduction system and on the skeletal muscle electrical system, the role of the NO system in this K+ - related electrical activity. And importantly, before furosemide or alternatively, as a therapy, after furosemide, when hypokalemia-third-degree AV block and ventricular tachycardia were already established, the beneficial effects obtained suggest potentially effective therapeutic interventions (null mortality, BPC 157>L-arginine) for acute hypokalemia. L-NAME/L-arginine mutual counteraction ascertains full accuracy for NO-system effects and involvement [21]. Furthermore, BPC 157 more powerful than L-arginine (i.e., arrhythmias and myoclonus elimination (BPC 157) *vs.* attenuation (L-arginine), particularly evident when each of

90 min after furosemide, before *intraperitoneal* medication		Medication at 90 min after furosemide	Time after furosemide (post-furosemide time) (min)						
			95	100	110	120	130	140	150
			Time after medication application (post-therapy time) (min)						
ECG amplitudes of P, R, S, T waves (0.01mV); P wave duration, QRS, QT intervals duration (msec)			5	10	20	30	40	50	60
P wave amplitude (0.01mV)	3.5±0.3	CONTROL(saline 5ml/kg i.p.)	3.3± 0.3	3.5± 1.2	3.4± 0.9	3.5± 1.1	3.6± 1.3	3.6± 1.2	3.4± 1.3
	3.3±0.1	BPC 10µg/kg i.p.	2.6± 0.15	2.7± 0.2	2.7± 0.9	2.8± 1.3	2.9± 0.4	2.9± 0.8	2.5± 1.1
	3.3±0.2	BPC 10ng/kg i.p.	2.4± 0.3	2.6± 0.2	2.7± 0.7	2.7± 1.1	2.8± 0.9	2.8± 1.4	2.6± 1.1
R wave amplitude (0.01mV)	33.2±0.7	CONTROL(saline 5ml/kg i.p.)	32.18± 0.5	33.2± 0.7	33.4± 0.3	32.5± 0.5	32.1± 0.7	32.6± 0.8	35.7± 1.7
	32.3±0.4	BPC 10µg/kg i.p.	40.9± 0.4 †	41.4± 1.2 †	41.1± 1.1 †	41.6± 1.2 †	41.4± 1.3 †	41.5± 1.5 †	42.5± 1.6 †
	32.5±0.6	BPC 10ng/kg i.p.	41.4± 0.5 †	41.7± 0.6 †	41.3± 1.2 †	41.5± 1.4 †	41.4± 1.4 †	41.4± 0.9 †	43.2± 1.2 †
S wave amplitude (0.01mV)	14.1±0.6	CONTROL(saline 5ml/kg i.p.)	14.6± 0.9	15.2± 0.9	15.1± 0.7	15.2± 0.3	15.6± 1.1	15.3± 1.2	12.6± 2.6
	14.3±0.2	BPC 10µg/kg i.p.	22.2± 0.7 †	20.9± 0.4 †	21.5± 1.1 †	22.7± 1.3 †	23.2± 1.5 †	23.6± 1.7 †	23.1± 1.4 †
	13.9±0.5	BPC 10ng/kg i.p.	22.2± 0.5 †	20.9± 0.5 †	21.1± 0.9 †	21.3± 1.1 †	21.4± 0.4 †	21.5± 0.9 †	23.2± 1.6 †
T wave amplitude (0.01mV)	1.3± 0.1	CONTROL(saline 5ml/kg i.p.)	1.4± 0.2	1.3± 0.1	1.2± 0.9	1.2± 1.1	1.2± 0.7	1.2± 0.3	1.3± 0.3
	1.3± 0.3	BPC 10µg/kg i.p.	2.2± 0.4 †	2.3± 0.4 †	2.3± 0.8 †	2.4± 0.1 †	2.4± 0.6 †	2.4± 0.5 †	2.4± 0.2 †
	1.4± 0.1	BPC 10ng/kg i.p.	2.5± 0.1 †	2.4± 0.2 †	2.3± 0.1 †	2.2± 0.2 †	2.4± 0.4 †	2.3± 0.3 †	2.4± 0.1 †
P wave duration (msec)	23.5± 0.8	CONTROL(saline 5ml/kg i.p.)	22.6± 0.4	23.5± 0.3	23.4± 0.4	23.9± 0.9	23.6± 1.1	24.3± 0.8	24.7± 1.2
	22.7± 0.4	BPC 10µg/kg i.p.	18±0.6 †	18.1±0.1 †	17.8±0.4 †	18.1±0.1 †	17.2± 1.1 †	18.3±0.4 †	18.3± 0.4 †
	23.4± 0.4	BPC 10ng/kg i.p.	18.8± 0.3 †	19.1± 0.2 †	19.1± 0.4 †	19.2±0.6 †	19.3± 0.9 †	19.2± 0.7 †	19.1± 1.1 †
QRS interval duration (msec)	30.3± 0.6	CONTROL(saline 5ml/kg i.p.)	31.5± 0.8	31.8± 0.4	32.4± 1.1	32.7± 0.9	32.7± 0.2	32.5± 0.6	33.7± 0.4
	31.0± 0.4	BPC 10µg/kg i.p.	14.4± 1.1 †	14.6± 0.3 †	14.8± 0.4 †	14.9± 0.7 †	14.8± 0.4 †	14.7± 0.4 †	14.5± 0.2 †
	33.1± 0.8	BPC 10ng/kg i.p.	14.4± 0.3 †	14.2± 0.4 †	14.5± 0.4 †	14.4± 0.7 †	14.2± 0.3 †	14.1± 0.6 †	14.1± 0.4 †
QT interval duration (msec)	103.7± 2.2	CONTROL(saline 5ml/kg i.p.)	101.7± 1.8	102± 0.9	101.9± 0.4	101.7± 0.8	101.5± 0.2	101.6± 0.1	101.5± 0.6
	101.5± 1.9	BPC 10µg/kg i.p.	68.4± 0.78 †	70.7± 1.5 †	70.1± 1.1 †	70.2± 0.9 †	70.1± 0.4 †	69.8± 1.1 †	68.8± 0.8 †
	102.2± 1.7	BPC 10ng/kg i.p.	69.4± 1.0 †	71.5± 1.1 †	70.8± 1.4 †	70.1± 1.3 †	70.2± 0.9 †	71.1± 0.8 †	71.4± 1.2 †

Table 3B: Effects of hypokalemia on amplitudes (0.01mV) of ECG waves, ECG wave duration and interval duration (msec). BPC 157 administered intraperitoneally as a therapy, after furosemide (100 mg/kg intraperitoneally), when hypokalemia- third-degree AV block and ventricular tachycardia were already established. Values are mean ± SEM. †P<0.05 significantly different from control values.

them was combined with L-NAME), alike BPC 157>L-arginine life-saving potential in doxorubicine-intoxication [9], methyldigoxin-intoxication, methyldigoxin-L-NAME-intoxication [10] all together generalize the same life-saving dose regimen (µg-ng) and route of application (intraperitoneal, intragastrical), and prophylactic and therapy protocols; both early development, and late advanced stage of doxorubicine-, methyldigoxin-intoxication and hypokalemia-syndrome. Also, BPC 157>L-arginine, BPC 157 effects not exaggerated by that of L-arginine [10,13,14] may represent distinctive ways in NO-alternative rescuing system. Finally, to prove possible general mechanism how BPC-157 could protect cells in hypokalemic conditions we used HEK293 cell. Repeating hypokalemic step in the presence of BPC-157, cells did not hyperpolarized. After washing BPC-157 from bath solution, under hypokalemic conditions cells were less hyperpolarized.

Before furosemide and before *intragastric* medication		Medication at 15 min before furosemide	Time after furosemide (post-furosemide time) (min)						
			95	100	110	120	130	140	150
			Time after medication application (post-therapy time) (min)						
Amplitudes of ECG P, R, S, T waves (0.01mV); P wave duration, QRS, QTintervals duration (msec)			110	115	125	135	145	155	165
P wave amplitude (0.01mV)	2.8± 1.0	CONTROL(saline 5ml/kg i.g.)	3.0± 0.6	3.1± 1.3	3.2± 0.9	3.3± 1.0	3.3± 1.4	3.4± 1.1	3.4± 1.6
	2.8± 1.4	BPC 10µg/kg i.g.	2.7± 1.0	2.8± 1.7	2.9± 1.1	2.9± 1.2	2.9± 1.1	2.9± 1.9	3.1± 1.3
	2.7± 0.9	BPC 10ng/kg i.g.	2.8± 0.4	2.9± 1.4	2.9± 0.9	2.9± 0.7	2.9± 1.6	2.9± 1.8	3.0± 1.8
R wave amplitude (0.01mV)	57.2± 1.9	CONTROL(saline 5ml/kg i.g.)	38.9± 2.7	37.9± 3.1	37.7± 2.4	35.2± 3.2	35.1± 2.7	34.9± 2.5	34.4± 2.1
	58.4± 1.6	BPC 10µg/kg i.g.	52.9± 2.4 †	53.2± 2.3 †	52.2± 1.8 †	51.9± 2.4 †	52.2± 1.9 †	52.9± 2.7 †	51.6± 2.7 †
	57.9± 1.5	BPC 10ng/kg i.g.	52.8± 2.7 †	54.4± 2.1 †	53.5± 1.9 †	53.7± 1.7 †	53.3± 1.6 †	53.8± 2.3 †	52.6± 2.1 †
S wave amplitude (0.01mV)	22.6± 1.5	CONTROL(saline 5ml/kg i.g.)	13.5± 1.8	13.7± 2.1	13.5± 1.2	12.2± 2.4	12.8± 2.4	12.0± 2.7	12.2± 2.1
	22.1± 1.2	BPC 10µg/kg i.g.	22.7± 1.2 †	23.1± 1.1 †	22.6± 1.6 †	22.7± 1.3 †	23.1± 1.2 †	23.0± 1.2 †	23.0± 0.9 †
	22.5± 1.6	BPC 10ng/kg i.g.	22.1± 1.4 †	23.8± 1.3 †	22.9± 1.7 †	22.6± 1.4 †	23.5± 1.3 †	23.4± 1.6 †	23.1± 1.3 †
T wave amplitude (0.01mV)	2.2± 0.9	CONTROL(saline 5ml/kg i.g.)	1.5± 0.8	1.5± 1.1	1.4± 1.2	1.4± 1.3	1.4± 1.5	1.4± 1.1	1.4± 0.9
	2.1± 0.7	BPC 10µg/kg i.g.	2.4± 1.1 †	2.3± 1.2 †	2.3± 0.9 †	2.5± 1.2 †	2.5± 1.3 †	2.5± 1.1 †	2.6± 0.9 †
	2.2±1.0	BPC 10ng/kg i.g.	2.2± 0.8 †	2.2± 1.3 †	2.3± 1.4 †	2.4± 1.6 †	2.3± 1.1 †	2.4± 1.5 †	2.4± 1.4 †
P wave duration (msec)	17.2±1.1	CONTROL(saline 5ml/kg i.g.)¥	25.3± 1.8	25.9± 1.4	26.3± 1.4	26.7± 1.8	26.9± 1.1	27.4± 2.1	27.9± 1.7
	17.4±1.4	BPC 10µg/kg i.g¥	22.4± 1.5	18.2± 1.1 †	17.4± 1.1 †	17.3± 1.7 †	17.1± 1.9 †	17.7± 1.6 †	18.0± 1.9 †
	17.1±1.6	BPC 10ng/kg i.g.¥	22.9± 1.4	19.0± 1.6 †	18.7± 2.1 †	18.4± 1.2 †	18.2± 1.4 †	18.7± 1.4 †	18.9± 2.3 †
QRS interval duration (msec)	16.6±1.3	CONTROL(saline 5ml/kg i.g.)¥	31.1± 2.1	31.9± 2.2	32.4± 2.6	32.7± 1.5	33.1± 1.5	33.8± 1.9	34.1± 1.8
	16.7±1.2	BPC 10µg/kg i.g.¥	29.9± 1.9	18.2± 1.7 †	18.0± 2.1 †	17.5± 1.7 †	17.3± 1.4 †	17.1± 1.5 †	17.2± 1.7 †
	16.3±1.7	BPC 10ng/kg i.g.	30.1± 2.1	18.8± 1.7 †	18.5±1.9 †	18.1± 2.1 †	17.9± 1.2 †	17.7± 1.1 †	17.4± 1.1 †
QT interval duration (msec)	81.6±2.1	CONTROL(saline 5ml/kg i.g.)¥	104.2± 2.1	104.7± 1.3	104.9± 2.1	104.9± 2.9	105.3± 1.9	105.7± 2.1	105.8± 1.4
	83.2±2.7	BPC 10µg/kg i.g.¥	103.9± 2.7	85.3± 2.1 †	84.3± 1.9 †	83.2± 2.2 †	83.1± 1.7 †	83.4± 2.8 †	82.7± 1.7 †
	83.9±2.4	BPC 10ng/kg i.g.¥	104.4± 2.2	88.3± 2.5 †	87.7± 1.4 †	86.7± 2.7 †	85.4± 2.9 †	85.1± 2.1 †	83.5± 2.2 †

Table 4A: Effects of hypokalemia on amplitudes (0.01mV) of ECG waves, ECG wave duration and interval duration (msec). BPC 157 administered intragastrically was given prophylactically before furosemide (100 mg/kg intraperitoneally). Values are mean ± SEM. ¥ - Two-way ANOVA with repeated measure on one factor ($P<0.05$) difference between measurements depends on group membership.†$P<0.05$ significantly different from control values.

However, despite all these arguments, the same hypokalemia in all furosemide-groups while different outcome further question to how BPC 157, L-arginine and L-NAME specifically worked. This could be not simply explained by the regular furosemide action, inhibition of Na-K ATPase activity in the medullar thick ascending limb of Henle's loop [7] or increased distal tubular fluid flow, or aldosterone, catecholamine and antidiuretic hormones actions that could contribute to the increase in potassium secretion whatever they are working maximally, or were already exhausted. But, any feedback from the renal part, less likely to have direct influence (i.e., generally averted furosemide-forced diuresis, decreased by L-NAME, postponed by L-arginine and BPC 157) should be NO-system related (L-NAME is antagonized by L-arginine or BPC 157).

Furthermore, "same hypokalemia"-response could be discriminative. With accelerated death, it requires less diuresis in L-NAME-furosemide rats, NOS-blockade and disable NO-system, L-NAME-furosemide rats being more susceptible to hypokalemia and hypokalemia-cardiac and extra-cardiac disturbances; while with no mortality of L-arginine- and BPC 157-rats could sustain more diuresis with considerably better outcome. For instance, AV block and abnormal ventricular rhythm either did not appear (i.e., BPC 157+furosemide) or sinus rhythm completely rescued within a few minutes (i.e.,

90min after furosemide before *intragastric* medication		Medication at 90 min after furosemide	Time after furosemide (post-furosemide time) (min)						
			95	100	110	120	130	140	150
			Time after medication application (post-therapy time) (min)						
ECG amplitudes of P, R, S, T waves (0.01mV); P wave duration, QRS, QT, RR intervals duration (msec)			5	10	20	30	40	50	60
P wave amplitude (0.01mV)	3.2±0.6	CONTROL(saline 5ml/kg i.g.)	3.1± 0.8	3.3± 0.7	3.2± 0.6	3.6± 0.9	3.5± 1.1	3.5± 1.0	3.5± 0.8
	3.1±0.4	BPC 10µg/kg i.g	3.2± 0.3	3.1± 0.4	2.9± 0.8	3.1± 0.7	3.2± 0.6	3.3± 0.7	3.1± 1.0
	3.4±0.7	BPC 10ng/kg i.g	3.2± 0.6	3.0± 0.3	3.1± 0.5	3.2± 0.8	3.3± 0.9	3.2± 0.9	3.2± 0.7
R wave amplitude (0.01mV)	32.9±0.6	CONTROL(saline 5ml/kg i.g.)	32.5± 0.9	34.1± 0.3	34.3± 0.6	33.6± 0.4	33.6± 0.8	33.1± 0.3	34.4± 0.9
	32.7±0.9	BPC 10µg/kg i.g	36.2± 0.7	42.4± 1.3 †	42.7± 0.8 †	41.9± 1.3 †	43.5± 1.1 †	43.8± 1.7 †	45.1± 1.8 †
	33.1±0.8	BPC 10ng/kg i.g	34.2± 0.9	42.1± 0.9 †	42.2± 0.9 †	42.5± 1.1 †	42.8± 0.9 †	43.7± 1.4 †	44.7± 1.3 †
S wave amplitude (0.01mV)	15.2±0.2	CONTROL(saline 5ml/kg i.g.)¥	15.3± 1.1	15.1± 0.5	16.1± 0.4	15.9± 0.6	15.6± 1.1	15.3± 1.2	12.6± 2.6
	14.9±0.5	BPC 10µg/kg i.g¥	15.6± 0.9	21.1± 0.7 †	21.8± 1.2 †	22.2± 1.1 †	23.2± 1.5 †	23.6± 1.7 †	23.1± 1.4 †
	15.3±0.8	BPC 10ng/kg i.g¥	15.9± 0.7	21.1± 0.4 †	21.3± 1.6 †	21.9± 1.0 †	21.4± 0.4 †	21.5± 0.9 †	23.2± 1.6 †
T wave amplitude (0.01mV)	1.3±0.4	CONTROL(saline 5ml/kg i.g.)¥	1.3± 0.1	1.4± 0.8	1.4± 0.1	1.3± 0.9	1.3± 0.9	1.3± 0.1	1.3± 0.2
	1.3±0.1	BPC 10µg/kg i.g¥	2.1± 0.9†	2.3± 0.7 †	2.3± 0.8 †	2.4± 0.1 †	2.4± 0.9 †	2.4± 0.1 †	2.5± 0.1 †
	1.4±0.1	BPC 10ng/kg i.g¥	2.2± 1.3†	2.5± 0.7 †	2.5± 0.3 †	2.4± 0.2 †	2.4± 0.1 †	2.3± 0.4 †	2.3± 0.7 †
P wave duration (msec)	22.9±1.2	CONTROL(saline 5ml/kg i.g.)	22.4± 1.2	23.1± 1.3	23.7± 1.3	23.9± 1.8	23.5± 1.4	23.9± 1.2	24.0± 1.1
	23.1±1.4	BPC 10µg/kg i.g	22.9± 1.6	18.6± 1.2 †	18.3± 1.1 †	18.5± 1.3 †	18.9± 1.3 †	18.5± 1.4 †	18.8± 1.4 †
	23.6±1.6	BPC 10ng/kg i.g	23.1± 1.2	18.9± 1.2 †	19.0± 1.4 †	19.2± 1.5 †	19.0± 1.1 †	19.1± 1.7 †	19.3± 1.3 †
QRS interval duration (msec)	31.7±1.4	CONTROL(saline 5ml/kg i.g.)	31.5± 0.8	32.2± 1.4	32.1± 1.6	32.7± 1.4	32.1± 1.2	32.5± 1.6	33.3± 1.4
	32.1±1.3	BPC 10µg/kg i.g	31.9± 1.4	18.5± 1.7 †	17.9± 1.2 †	17.7± 1.7 †	17.3± 1.4 †	17.1±1.3 †	17.5± 1.3 †
	31.5±1.4	BPC 10ng/kg i.g	31.8± 1.3	19.1± 1.8 †	18.5± 1.4 †	18.2± 1.5 †	18.3± 1.5 †	17.9± 1.6 †	18.1± 1.5 †
QT interval duration (msec)	102.9±1.9	CONTROL(saline 5ml/kg i.g.)¥	102.7± 1.5	102.4± 1.8	102.8± 1.2	102.7± 1.4	102.5± 2.1	101.9± 2.1	102.1± 1.5
	102.3±2.1	BPC 10µg/kg i.g¥	99.2± 1.9 †	76.5± 1.8 †	76.2± 1.6 †	75.9± 1.6 †	75.7± 1.6 †	76.2± 1.8 †	76.3± 1.1 †
	102.8±1.8	BPC 10ng/kg i.g¥	99.4± 1.5 †	77.2± 1.5 †	77.5± 1.7 †	77.3± 1.8 †	76.7± 2.1 †	76.4± 1.7 †	76.1± 1.9 †

Table 4B: Effects of hypokalemia on amplitudes (0.01mV) of ECG waves, ECG wave duration and interval duration (msec). BPC 157 administered intragastrically, as a therapy, after furosemide (100 mg/kg intraperitoneally), when hypokalemia- third-degree AV block and ventricular tachycardia were already established. Values are mean ± SEM. ¥ - Two-way ANOVA with repeated measure on one factor (P<0.05) difference between measurements depends on group membership. †P<0.05 significantly different from control values.

furosemide+BPC 157)) provide a normal ECG presentation and survival despite steady severe hypokalemia in furosemide-rats. Counteraction of AV blocks (including third-degree AV block) occurs no matter what the total absence of AV conduction (in digitalis-intoxication [10] or AV conduction block (including third-degree AV block) was in Hiss-Purkinje system in furosemide-hypokalemic rats. Note, in hypokalemia, decrease of conduction velocity and prolongation of relative refractory period are more expressed in Hiss-Purkinje fibers than in ventricular fibers along with commonly thought hypokalemia-induced abnormal pacemaker activity attributed to increased slope of diastolic depolarization in Purkinje fibers [22,23].

Thus, besides the counteraction of hypokalemic disturbances in

Medication at 15 min before furosemide	Rhythm	RR interval duration (msec) before furosemide medication (per 5 min.)	RR interval duration (msec) after furosemide						
			Time after furosemide (post-furosemide time) (min)						
			95	100	110	120	130	140	150
			Time after medication application (post-therapy time) (min)						
			110	115	125	135	145	155	165
CONTROL(saline 5ml/kg i.p.) ¥	sinus rhythm	177±2.1	201±1.1	201.8±1.9	200.2±1.7	201.7±2.2	201.9±2.1	201.6±2.4	201.7±2.1
	ventricular tachycardia	-	293.2±3.5	286.7±4.1	299.2±2.4	273.4±2.5	288±3.1	288.4±3.7	285.3±1.7
	third-degree AV block	-	99.4±2.1	101.4±2.6	97.3±3.7	95.6±2.5	100.5±1.8	97.8±1.6	93.6±4.1
BPC 10µg/kg i.p. ¥	sinus rhythm	188±2.1	160±2.1†	168.1±2.†	167.9±2.†	166.9±2.†	169.1±1.†	169.8±2.†	172.2±2†
	ventricular tachycardia	-	-	-	-	-	-	-	-
	third-degree AV block	-	-	-	-	-	-	-	-
BPC 10ng/kg i.p. ¥	sinus rhythm	181±1.5	170±1.9 †	175.2±2.2†	174.1±2.1†	171.1±2.3†	177.1±1.9†	178.3±1.9†	179.5±27†
	ventricular tachycardia	-	-	-	-	-	-	-	-
	third-degree AV block	-	-	-	-	-	-	-	-
CONTROL(saline 5ml/kg i.g.) ¥	sinus rhythm	180.2±2.8	203.7±2.1	202.9±2.2	202.5±1.9	202.2±2.6	202.0±2.8	202.3±2.1	201.9±2.6
	ventricular tachycardia	-	299.1±4.1	293.4±3.8	287.3±3.7	288.1±2.5	289.1±3.1	293±4.1	295.3±2.8
	third-degree AV block	-	99.1±3.4	101.5±2.6	97.8±2.2	98.8±2.5	96.4±3.1	94.5±2.8	92.1±4.1
BPC 10µg/kg i.g. ¥	sinus rhythm	185.2±2.4	202.6±1.9	176.4±2.6†	175.8±2.7†	175.1±2.9†	174.3±2.1†	174.9±2.4†	173.5±2.1 †
	ventricular tachycardia	-	-	-	-	-	-	-	-
	third-degree AV block	-	-	-	-	-	-	-	-
BPC 10ng/kg i.g. ¥	sinus rhythm	183.9±1.9	203.1±2.5	179.8±2.1†	179.2±2.8†	179.0±2.7†	178.8±2.2†	178.4±1.7†	178.1±2.2 †
	ventricular tachycardia	-	-	-	-	-	-	-	-
	third-degree AV block	-	-	-	-	-	-	-	-

Table 5A: Effects of hypokalemia on RR interval duration (msec) during sinus rhythm, ventricular tachycardia, third-degree AV block. BPC 157 (intraperitoneally or intragastrically) was given prophylactically before furosemide (100 mg/kg intraperitoneally). Values are mean ± SEM. ¥ - Two-way ANOVA with repeated measure on one factor (P<0.05) difference between measurements depends on group membership.†P<0.05 significantly different from control values.

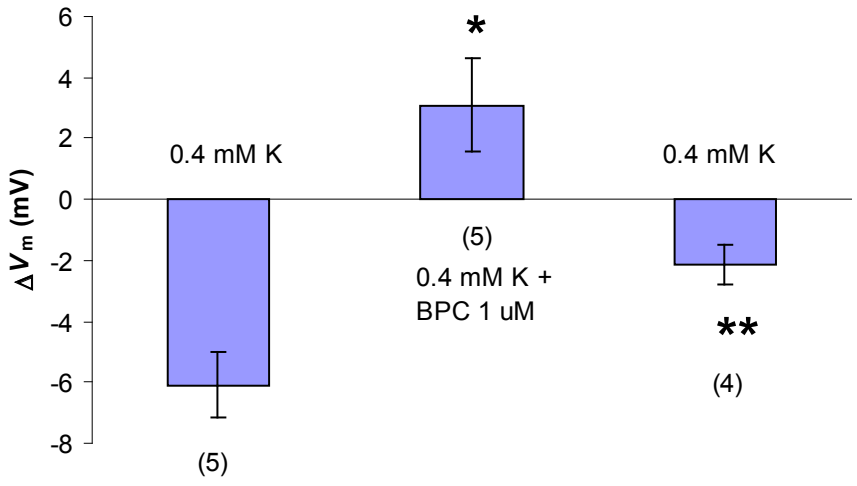

Figure 1: BPC-157 (1 uM) abolished hyperpolarizationsof HEK293 cells during hypokaliemicconditions. After washing BPC-157 out, hyperpolarizationsresponse on hypokaliemiarecovered. Data represent the mean ± SE with the number of experiments given in brackets (paired experiments). P < 0.05 compared effects of hypokaliemicconditions on the membrane voltages (V_m) with and without presence of BPC-157, ** P < 0.05 effects of hypokaliemiaon mafter washing BPC-157 out compared to changes of V_m under hyokaliemicconditionsin the presence of BPC-157

Rhythm	RR interval duration (msec) after furosemide and before medication (per 5 min.)	Medication at 90 min after furosemide	Time after furosemide (post-furosemide time) (min)						
			95	100	110	120	130	140	150
			Time after medication application (post-therapy time) (min)						
			5	10	20	30	40	50	60
sinus rhythm	193.9±3.1	**CONTROL (saline 5ml/kg i.p.) ¥**	191.3±2.1	195±1.7	196.2±1.4	196.4±2.4	197.9±1.4	198.4±2.1	199.9±1.5
ventricular tachycardia	272.6±3.6		285.3±3.4	273.9±4.1	288.1±3.7	289.3±2.7	277.9±3.2	283.5±3.9	281.8±4.1
third-degree AV block	107±2.7		101.2±2.5	99.8±3.1	104.6±2.5	101.5±4.1	97.3±2.8	96.3±3.5	92.7±5.6
sinus rhythm	194.4±2.9	**BPC 10µg/kg i.p. ¥**	155.5±1.4†	160±2.5 †	157±2.1 †	166±1.7 †	165±0.8 †	162±0.9 †	161.2±1.4†
ventricular tachycardia	269.1±2.7		263.3±2.1	-	-	-	-	-	-
third-degree AV block	98.2.1±3.2		97.2±4.1	-	-	-	-	-	-
sinus rhythm	194.3±2.8	**BPC 10ng/kg i.p. ¥**	153.4±1.6†	162±3.6 †	164.1±2.7†	161.1±2.3†	162.1±2.3†	161.1±1.9†	161.2±2.2†
ventricular tachycardia	272.3±3.1		273.8± 2.6	-	-	-	-	-	-
third-degree AV block	98.6±2.1		99.1±2.6	-	-	-	-	-	-
sinus rhythm	195.7±2.6	**CONTROL (saline 5ml/kg i.g.) ¥**	194.7±2.4	195.1±1.2	195.5±1.8	196.1±1.9	196.5±2.5	197.1±1.7	198.1±2.1
ventricular tachycardia	295.2±2.9		294±3.7	287±5.1	269.2±3.1	286.3±3.4	299.1±2.1	285.7±4.1	279.9±2.9
third-degree AV block	101.7±2.4		104.2±2.5	99.4±2.1	96.3±3.6	100.2±2.2	97.8±3.6	94.3±5.1	92.2±3.9
sinus rhythm	196.1±2.1	**BPC 10µg/kg i.g. ¥**	195.7±2.2	169.1±1.9†	169.4±2.2†	169.6±2.3†	167.9±1.7†	167.7±1.6†	167.4±1.9 †
ventricular tachycardia	289.2±1.9		285.4±2.1	266.7±2.1	-	-	-	-	-
third-degree AV block	97.4±2.6		99.7±3.6	104.3±1.8	-	-	-	-	-
sinus rhythm	195.9±2.3	**BPC 10ng/kg i.g. ¥**	194.1±1.5	170.3±1.7†	171.1±2.3†	170.8±2.8†	170.1±1.4†	170.0±1.1†	169.4±1.8 †
ventricular tachycardia	289.3±2.5		294.5±4.1	288.1±2.6	-	-	-	-	-
third-degree AV block	98.4±2.1		101.6±3.1	99.4±2.7	-	-	-	-	-

Table 5B: Effects of hypokalemia on RR interval duration (msec) during sinus rhythm, ventricular tachicardia, third-degree AV block. BPC 157 (intraperitoneally or intragastrically) as a therapy, after furosemide (100 mg/kg intraperitoneally), when hypokalemia- third-degree AV block and ventricular tachycardia were already established. Values are mean ± SEM. ¥ - Two-way ANOVA with repeated measure on one factor (P<0.05) difference between measurements depends on group membership. †P<0.05 significantly different from control values.

Medication at 90 min after furosemide	The rats were monitored throughout the 60 min after medication application					
	Number of premature ventricular beats (per min), Mean±S.D.	Duration of ventricular tachycardia (min), Mean±S.D.	Duration of third-degree AV block (min), Mean±S.D.	Absolute termination of arrhythmias (min.), Mean±S.D.	Time till fatal outcome (min.), Mean±S.D.	Mortality rate (surviving/dead rats)
CONTROL(saline 5ml/kg i.p.)	104.1±3.6	8.3±2.1	6.8±1.5	Not determined	55.6±3.8	0/10
BPC 10µg/kg i.p.	14.6±4.9 †	0.5±0.1 †	0.6±0.2 †	4.9±0.1 †	Not determined	10/0 †
BPC 10ng/kg i.p.	18.2±3.2 †	0.8±0.1†	1.0±0.3†	7.9±0.2 †	Not determined	10/0 †
CONTROL(saline 5ml/kg i.g.)	106.3±2.9	11.3±3.6	9.6±2.1	Not determined	52.9±5.9	0/10
BPC 10µg/kg i.g.	19.2±3.9 †	0.8±0.4 †	0.8±1.1†	8.9±0.2 †	Not determined	10/0 †
BPC 10ng/kg i.g.	25.7±4.2 †	1.1±0.6 †	1.2±0.8 †	11.2±0.3†	Not determined	10/0†

Table 6: When hypokalemia- third-degree AV block and ventricular tachycardia were already established, rats were monitored throughout the 60 min after application BPC 157 (intraperitoneally or intragastrically) as a therapy at 90 min after furosemide for a number of premature ventricular beats (per min), duration of ventricular tachycardia (min), duration of third-degree AV block (min), absolute termination of arrhythmias (min.), time till fatal outcome (min.), mortality rate (surviving/dead rats). Values are mean ± SEM. †P<0.05 significantly different from control values.

vitro, and thereby a direct effect on membrane, the "same hypokalemia"-question may be solved via targeting cardiac directly (i.e., BPC 157's beneficial effect on myocardial muscle and conductive tissue was suggested also in doxorubicine- and methyldigoxin-intoxication) [9,10] or vasculature. Obviously, that effect should be multifactorial and different from the standard antiarrhytmic agents [9,10]. For instance, BPC 157 therapy likely involves a counteraction of downregulation of IKr potassium current (no consequence of the reduced extracellular potassium-paradoxical inhibition of the activity of the IKr potassium current [24] and delayed ventricular repolarization), counteracted suppression of conduction (counteracted prolongation of the PQ and QRS, RR interval), and increased refractoriness also counteracted

The ECG rats were monitored throughout the 60 min, after reaching a severe hypokalemia at 90min after administration of furosemide. Medication given 15 min before furosemide.							
REGIMEN	**STUDY GROUPS**	Number of premature ventricular beats (per 60 min), Mean±S.D.	Duration of ventricular tachycardia (min), Mean±S.D.	Duration of third-degree AV block (min), Mean±S.D.	Absolute termination of arrhythmias (min), Mean±S.D.	Time till fatal outcome (min), Mean±S.D.	Mortality rate (surviving/ dead rats)
PROPHYLACTIC	CONTROL (SALINE 5ml/kg)	103.4±4.2	19.8±2.7	8.9±1.8	0	57.3±4.2	0/10
	BPC 157	2.1±0.1 †	0 †	0 †	-	Not determined	10/0 †
	L-NAME	156±5.6 †	11.9±3.6 †	25.7±4.1 †	0	31.2±1.5 †	0/10
	L-NAME + BPC 157	128.2±3.7 †	7.9±2.1 †	3.1±0.8 †	9.2±2.3 †	Not determined	10/0 †
	L-arginine	100.8±2.9	17.9±1.5	7.5±1.5	26.3±3.1 †	Not determined	10/0 †
	L-arginine + BPC 157	42.7±5.8 †	4.5±1.4 †	1.2±0.4 †	5.3±3.7 †	Not determined	10/0 †
	L-NAME + L-arginine	108.3±5.1	18.5±2.3	9.4±2.1	0	59.6±3.4	0/10
	L-NAME + L-arginine + BPC 157	52.9±6.1 †	0 †	0 †	-	Not determined	10/0 †

Table 7A: Development of furosemide-induced hypokalemic arrhythmias after application of pentadecapeptide BPC 157 (10 µg/kg i.p.), L-NAME (5 mg/kg i.p.), inhibitor of NO-synthase, and L-arginine (100 mg/kg i.p.), substrate of NO-synthase and NO-precursor – PROPHYLACTIC REGIMEN (medication given 15 min. before furosemide). +P≤0.05 at least vs. control.

The ECG rats were monitored throughout the 60 min after application of the medicaments. Medication given 90 min. after furosemide									
REGIMEN	**STUDY GROUPS**	**Number of premature ventricular beats), Mean±S.D.**		**Duration of ventricular tachycardia (min), Mean±S.D.**		**Duration of third-degree AV block (min), Mean±S.D.**		**Absolute termination of arrhythmias (min.), Mean±S.D.**	**Time till fatal outcome (min.), Mean±S.D.**	**Mortality rate (surviving/ /dead rats)**
THERAPEUTIC		**BASELINE before medication (per 5min)**	**5–60 min. after medication**	**BASELINE before medication (per 5 min)**	**5–60 min after Medication**	**BASELINE before medication (per 5 min.)**	**5–60 min after medication**			
	CONTROL (SALINE 5ml/kg)	11.7±3.1	104.1±3.6*¥	0.7±0.3	18.3±2.1*¥	0.8±0.4	6.8±1.5*¥	Not determined	55.6±3.8	0/10
	BPC 157	10.2±2.8	4.4±2.1†*¥	0.8±0.1	0 †*¥	0.6±0.2	0 †*¥	4.9±0.1 †	Not determined	10/0 †
	L-NAME	11.1±1.9	179.4±3.1†*¥	0.6±0.1	29.2±2.2†*¥	0.6±0.2	13.4±3.4†*¥	Not determined	25.4±3.6 †	0/10
	L-NAME + BPC 157	12.6±2.4	52.6±1.8†*¥	0.8±0.1	0.4±0.2†*¥	0.7±0.3	0.4±0.6 †¥	9.8±1.2 †	Not determined	10/0 †
	L-arginine	11.2±1.8	99.4±3.1*¥	0.6±0.4	16.9±1.8*¥	0.6±0.2	5.1±2.1 *¥	26.3±3.1 †	Not determined	10/0 †
	L-arginine + BPC 157	11.5±2.1	47.9±2.1†*¥	0.7±0.1	5.6±1.8†*¥	0.7±0.4	1.1±0.7 †¥	5.3±3.7 †	Not determined	10/0 †
	L-NAME + Larginine	11.6±2.2	113.2±3.7*¥	0.6±0.3	20.2±1.9*¥	0.8±0.1	10.7±2.9 *¥	Not determined	49.7±3.1	0/10
	L-NAME + Larginine + BPC 157	11.7±2.1	58.8±4.2†*¥	0.8±0.2	8.9±3.1†*¥	0.7±0.3	3.5±1.4†*¥	10.2±1.9	Not determined	10/0 †

Table 7B: The effect of pentadecapeptide BPC 157 (10 µg/kg i.p.), L-NAME (5 mg/kg i.p.), inhibitor of NO-synthase, and L-arginine (100 mg/kg i.p.), substrate of NO-synthase on the established furosemide-induced hypokalemic arrhythmias in the rat – THERAPEUTIC REGIMEN (Medication given 90 min. after furosemide). ¥ - Two-way ANOVA with repeated measure on one factor (P<0.05) difference between measurements depends on group membership. + P≤0.05 at least *vs.* control. * P≤0.05 at least vs. baseline.

(note, counteraction of prolongation of QT interval). Further - since no prolongation of the QT interval, and no abnormal ventricular rhythm appears, - variform ventricular tachycardia ("torsades de pointes") also antagonized means counteraction of early after depolarization and resultant triggering (thought to cause torsades de pointes syndrome [25,26]. Besides, the decrease of ECG wave amplitude (which does not necessarily mean that conduction is suppressed, and is not thought to be related to the degree of hypokalemia [7] was also counteracted. Note, BPC 157 beneficial effect [9,10] also includes hypoxic injury and reoxygenation arrhythmias [27]. What's more, presenting that hypokalemia [28] causes endothelial dysfunction and low NO levels, it is worth mentioning that BPC 157 maintains endothelium integrity [16,17] along with its effect on the NO-system and antagonization of LNAME effect [14]. Besides, although not directly related, other BPC 157 effects may also be indicative to prevent hypokalemia-disturbances. For instance, hypokalemia is important in insulin resistance development [29] while BPC 157 may also counteract insulin resistance [30].

Providing that potassium is essential for many body functions, including muscle and nerve activity [31] and the same essential rescuing role of NO-system in both heart [1,2] and skeletal muscle [32] a supportive analogy noted in the severely hypokalemic furosemide-

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REGIMEN	STUDY GROUPS	Total AV block (QRS complex duration, ventricular frequency)				Ventricular tachycardia(torsades de pointes) (QRS complex duration, heart frequency)			
THERAPEUTIC Medication at 90min after fursemide		**BASELINE before medication (per 5min.)**		**5–60 min. after medication**		**BASELINE before Medication (per 5 min.)**		**5–60 min. after medication**	
		QRS complex duration (msec)	**Ventricular frequency (beats/min)**	**QRS complex duration (msec)**	**Ventricular frequency (beats/min)**	**QRS complex duration (msec)**	**Ventricular frequency (beats/min)**	**QRS complex duration (msec)**	**Ventricular frequency (beats/min)**
	CONTROL (SALINE 5ml/kg)	31.7±1.4	99.4±2.1	32.1±1.3 ¥	93.6±4.1 ¥	30.9±1.8	289.8±4.1	34.4±2.4 ¥	284.1±3.6 ¥
	BPC 157 10µg/kg ip	32.1±1.3	97.2±4.1	23.9±2.1 †*¥	90.1±2.3 ¥	31.5±2.1	282.3±3.7	27.5±3.1 ¥	261.3±3.7 †*¥
	BPC157 10ng/kg	30±1.1	93±3.2	25±1.1*¥	95±2.5¥	33.1±1.1	292±4.0	24.5±2.0¥	250.1±2.1†*¥
	L-NAME	31.7±1.2	98.5±2.7	35.7±2.8 ¥	91.6±4.1 ¥	32.1±1.5	284.1±3.5	35.9±2.1 ¥	289.4±3.2 ¥
	L-NAME + BPC 157	31.9±1.5	99.2±3.2	27.3±1.8 †*¥	100.1±2.3 ¥	31.5±1.1	289.8±4.1	32.1±2.4 ¥	271.8±4.1 †*¥
	L-arginine	31.1±1.8	96.9±3.6	28.9±2.5¥	94.9±3.9 ¥	32.1±1.3	288.3±3.1	30.4±2.3 ¥	279.3±3.7 ¥
	L-arginine + BPC 157	31.8±1.4	97.6±2.1	22.9±1.9 †*¥	102.6±1.2 ¥	32.4±1.9	285.4±3.8	28.6±1.5 ¥	267.4±2.9 †*¥
	L-NAME + Larginine	32.1±1.3	99.5±2.7	23.2±3.1 †*¥	96.2±3.4 ¥	31.8±1.7	291.2±3.3	34.5±2.5 ¥	286.2±3.9 ¥
	L-NAME + Larginine + BPC 157	32.2±1.6	98.3±3.2	24.3±2.4 †*¥	102.3±2.5 ¥	31.9±2.3	289.3±2.9	30.1±2.8 ¥	269.3±3.7 †*¥
PROPHYLACTIC Medication at 15min before furosemide	**STUDY GROUPS**	**Total AV block (QRS complex duration. ventricular frequency)**				**Ventricular tachycardia(torsades de pointes) (QRS complex duration. heart frequency)**			
		BASELINE 90min after furosemide appplication		**95–150 min. after furosemide appplication**		**BASELINE 90min after furosemide appplication**		**95–1500 min. after furosemide appplication**	
		QRS complex duration (sec)	**Ventricular frequency (beats/min)**	**QRS complex duration (sec)**	**Ventricular frequency (beats/min)**	**QRS complex duration (sec)**	**Ventricular frequency (beats/min)**	**QRS complex duration (sec)**	**Ventricular frequency (beats/min)**
	BPC 157 10µg/kg	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A
	BPC 157 10ng/kg	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A
	L-NAME	35.7±1.8	99.4±1.4	42.1±2.2 ¥	86.6±5.3 ¥	38.9±1.8	296.8±1.1	48.4±2.4 ¥	277.1±5.6 ¥
	L-NAME + BPC 157	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A
	L-arginine	26.4.±1.5	112.4±4.1	36.1±2.1 ¥	105.6±6.2 ¥	40.9±1.8	304.8±6.1	41.1±3.3 ¥	296.1±3.1 ¥
	L-arginine + BPC 157	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A
	L-NAME + L-arginine	33.2±1.0	100.4±3.6	36.1±1.1 ¥	98.4±2.1 ¥	33.4±2.	296.2±3.5	38.0±2.0 ¥	288.1±5.0 ¥
	L-NAME + L-arginine + BPC 157	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A

Table 8: The effect of pentadecapeptide BPC 157 (10 µg/kg i.p.), L-NAME (5 mg/kg i.p.), inhibitor of NO-synthase, and L-arginine (100 mg/kg i.p.), substrate of NO-synthase on the interference interventricular implementation in furosemide-induced hypokalemic arrhythmias in the rat – THERAPEUTIC REGIMENT (Medication given 90 min. after furosemide). PROPHYLACTIC REGIMEN (Medication given at 15 min before furosemide). ¥ - Two-way ANOVA with repeated measure on one factor (P<0.05) difference between measurements depends on group membership. + P≤0.05 at least *vs.* control. * P≤0.05 at least vs. baseline.

REGIMEN	STUDY GROUPS	Absolute termination of arrhythmias (min) Mean±S.D.	Absolute termination of myoclonismus (min) Mean±S.D.
PROPHYLACTIC	CONTROL – saline	Not determined	Not determined
(Medication given at 15 min before furosemide)	BPC 157	No arrhythmias †	No myoclonus†
	L-NAME	Not determined	Not determined
	L-NAME + BPC 157	9.2±2.3 †	13.3±3.4 †
	L-arginine	26.3±3.1 †	18.6±2.1 †
	L-arginine + BPC 157	5.3±3.7 †	7.1±2.5 †
	L-NAME + L-arginine	Not determined	Not determined
	L-NAME + L-arginine + BPC 157	No arrhythmias †	No myoclonus†
THERAPEUTIC	CONTROL – saline	Not determined	Not determined
(Medication given at 90 min after furosemide)	BPC 157	4.9±0.1 †	9.5±2.5 †
	L-NAME	Not determined	Not determined
	L-NAME + BPC 157	13.3±2.2 †	19.9±4.1 †
	L-arginine	31.1±4.5 †	25.8±3.8 †
	L-arginine + BPC 157	10.5±2.9 †	13.5±3.1 †
	L-NAME + L-arginine	Not determined	Not determined
	L-NAME + L-arginine + BPC 157	8.2±1.9 †	11.2±3.4 †

Table 9: Absolute termination of furosemide-induced hypokalemic arrhythmias and myoclonismus after application of pentadecapeptide BPC 157 (10 µg/kg i.p.), L-NAME (5 mg/kg i.p.), inhibitor of NO-synthase, and L-arginine (100 mg/kg i.p.), substrate of NO-synthase and NO-precursor. †P<0.05 at least vs. control.

rats between cardiac and extra-cardiac manifestations (myoclonus) [8] presentation and therapy (L-NAME (aggravation) *vs.* L-arginine (attenuation), BPC 157 (elimination)) should be expected. This could be at the best perceived with respect to the analogous BPC 157 treatment effects; in prophylaxis and therapy (myoclonus either did not appear or severe myoclonus was very rapidly eliminated). Further, this analogy actually conveys in mentioned NO-system-terms cardiac muscle (decreased extracellular potassium: myocardial hyperexcitability with the potential to develop re-entrant arrhythmias) and skeletal muscle (decreased potassium levels in the extracellular space: hyperpolarization of the resting membrane potential, a greater than normal stimulus for depolarization of the membrane in order to initiate an action potential) [24]. In support, presenting the general significance of skeletal muscle for plasma potassium homeostasis [31], BPC 157's heart effect [9,10,27] are along with prominent neuroprotective effect [33,34], marked increase of the healing rate of severely damaged skeletal muscles and recovering of their function [35-37].

In summary, for potassium-related disturbances and BPC 157 antiarrhythmic and recovering effects, not only in theory, to obtain a successful recovery, besides interaction with NO-system that was now demonstrated [10-14], it could be essential to modify the negative chain of events. These include; the prematurely depolarized membrane, early after derpolarization, prolonged cardiac repolarization when extracellular K+ is low which provides polymorphic ventricular tachycardias and a long QT interval (i.e., "torsades de pointes"), which were all antagonized, are both, prevented (i.e., prophylactic regimens) and reversed (therapeutic regimens) by a single BPC 157 i.p./i.g. administration. Thus, no supplementation of potassium and/or cessation of the medications that induce hypokalemia clearly suggest therapeutic significance in acute hypokalemia for null mortality, BPC 157>L-arginine evidenced in this study.

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