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Differential stereoselective effects of levobupivacaine, bupivacaine and dexbupivacaine on the heart in isolated rat hearts

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Abstract

Bupivacaine (BUP) has an advantage over other local anesthetics because of its long-acting sensory anesthesia, but because of its high affinity for the myocardial Na^+ channel it can be cardiotoxic. Levobupivacaine (LBUP) is an S(−)-isomer of the racemate BUP, and dexbupivacaine (DBUP) is an R(+)-isomer. LBUP has less neuronal and cardiac toxicity than BUP and DBUP, but DBUP induces more negative chronotropism and arrhythmogenic effects than either LBUP or BUP.

We thus investigated the effects of LBUP, BUP and DBUP on the heart using isolated perfused rat hearts by measuring tissue ion contents. Using the Langendorff perfusion-system, each isolated rat heart was perfused with Krebs-Henseleit buffer containing various concentrations of LBUP, BUP and DBUP (0.5, 1.0, 5.0μg/ml) for 10 minutes. Thereafter, each heart was subjected to regional ischemia (11min) and reperfusion (3min). In other experiments using an atomic absorption spectrometer, we measured tissue Na^+, Ca^{2+}, and K^+ contents in rat heart.

There were significant differences in decreases of heart rate among the BUP formulations, showing reduction in the order LBUP > BUP > DBUP, and they had a protective effect against reperfusion-induced arrhythmias. In the experiment on the total tissue ion contents, significant differences were observed, particularly in Na^+ content, showing the reduction in the order LBUP > BUP > DBUP. It is suggested that there may be differences in Na^+ channel-blocking action, which affects conduction systems and protective effects against reperfusion-induced arrhythmias among the BUP formulations.

Key words; bupivacaine, levobupivacaine, dexbupivacaine, heart rate, ion

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Introduction

Bupivacaine (BUP) is an amide type, long-acting local anesthetic drug, and is currently used as a racemate consisting of an equimolar mixture of S(−)-levobupivacaine (LBUP) and R(+)-dexbupivacaine (DBUP). Stereospecificity has been observed in the pharmacological actions of LBUP, BUP and DBUP, and it has been reported that LBUP and BUP exert lower

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cardiotoxicity compared with DBUP[1,2]. Our previous studies[3,4] have also revealed that the effects on cardiac function differ among these three kinds of BUP formulations; LBUP was least effective in suppressing cardiac function, and the suppressed cardiac function could be alleviated by pacing or catecholamine. BUP, like lidocaine, a local anesthetic agent, blocks Na^+ and Ca^{2+} channels[5]. Although it has been speculated that actions on Na^+, Ca^{2+} and K^+ channels may differ among the BUP formulations[5], detailed comparative studies are scarce. In the present study, we examined the actions of the different modes of BUP formulation to the heart, by performing the experiment of reperfusion-induced arrhythmias in the isolated rat heart using Langendorff perfusion-system, and by determining the total tissue contents of Na^+, Ca^{2+} and K^+ ions using atomic absorption analysis.

Subjects and Methods

A. Experiment 1 : Effects of BUP formulations on reperfusion-induced arrhythmias

1. Subjects

In each group, 12 male rats of the Wistar ST strain, 9 to 10 weeks old, were used.

2. Experimental method (Fig. 1)

Cardiac perfusion was performed according to the method reported by Umeda, et al[6]. After etherization of the rats, the heart was isolated, then coronary perfusion was immediately started by the Langendorff method. Krebs-Henseleit buffer solution (mM: NaCl 118.5; $NaHCO_3$ 25; KCL 3.2; KH_2PO_4 1.19; $MgSO_4$ 1.18; $CaCl_2$ 2.5; glucose 11), saturated with 95% oxygen and 5% carbon dioxide and maintained at 37°C, was used as a perfusate, and was filtered (0.45μM: Millipore Corp.) before use. The Krebs-Henseleit buffer solution was placed in a perfusate cell, and a solution of 0.5, 1.0, or 5.0μg/ml levobupivacaine (Maruishi Pharmaceutical Co., Ltd.), bupivacaine (Sigma Chemical Co., Ltd.) or dexbupivacaine (Maruishi Pharmaceutical Co., Ltd.) in the Krebs-Henseleit buffer solution was placed in another perfusate cell.

The isolated rat heart was cannulated via the aorta and perfused at a constant perfusion pressure equivalent to 100cm of water. A ligature was then placed around the left ventricular branches of the circumflex artery. Both ends of the ligature were passed through a small plastic tube which was then passed against the artery. The electrodes for electrocardiography were then attached to the apex and aortic ostium to monitor and record the electrocardiogram (R-e-8710: POLYGRAPH, Sanei-Sokki Co., Ltd, Japan). After the heart rate and the coronary flow of the isolated heart were stabilized by perfusion with the Krebs-Henseleit buffer solution for 15 minutes, the perfusate was replaced by the Krebs-Henseleit buffer solution containing different concentrations of LBUP, BUP, or DBUP, and changes were observed for 10 minutes. After exposing the heart to an ischemic condition for 11 minutes, the clamp and the tube were removed, and then the heart was reperfused for 3 minutes. In the control group, cardiac perfusion was performed with the Krebs-Henseleit buffer solution alone.

3. Measurement items and statistical analysis

a. Measurement of coronary flow

Using a 25ml graduated cylinder, the coronary flow that was effluxed from the pulmonary artery was measured every 5 minutes for 1 minute before the respective measurement time points.

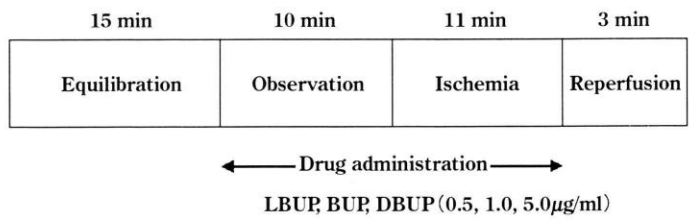

Figure 1 Experimental protocol

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b. Measurement of heart rate (HR) and analysis of reperfusion-induced arrhythmias

HR was measured every 5 minutes before the respective measurement time points. However, when the perfused heart showed no sinus rhythm after reperfusion, these measurements were not performed. Electrocardiogram was continuously recorded for up to 3 minutes after starting reperfusion, and the induced arrhythmias were analyzed according to the Lambeth Conventions guidelines[7], based on the eventually occurring ventricular fibrillation (VF), VF duration, ventricular tachycardia (VT), VT duration, and premature ventricular contraction (PVC). When VF occurred continuously after the 3-minute observation period, it was defined as sustained VF, and the VF duration was defined as the sum of the times VF occurred within 3 minutes after starting reperfusion.

c. Statistical analysis

The incidence rates of sustained VF, VF, VT and PVC were tested by Fisher's exact probability test. For the data analyses of coronary flow, HR, VF and VT durations, the paired Student t test and the Turkey-Kramer method were used for intra-group and inter-group significance tests, respectively, and the significance level was set to $P<0.05$.

B. Experiment 2: Effects of BUP formulations on the total tissue contents of Na^+, Ca^{2+} and K^+

1. Subjects

In each group, 6 male rats of the Wistar ST strain, 9 to 10 weeks old, were used.

2. Experimental method

Cardiac perfusion was performed in the same manner as in **Experiment 1**. The atomic absorption analysis for the isolated rat heart was performed according to the method reported by Takeo[8] and Kamiyama, et al.[9] The isolated rat heart was stabilized by perfusion with the Langendorff method, and then perfused with Krebs-Henseleit buffer solution containing 1.0 or 5.0μg/ml LBUP, BUP or DBUP for 10 minutes. After perfusion, the perfusate remaining in the heart was washed out by perfusion with a 10ml mixture of 320mM sucrose and 20mM Tris/HCl, adjusted to pH 7.4. The rat heart was dried at 180°C, and then incinerated at 180°C after adding 5ml concentrated nitric acid. After cooling, the resulting ash was diluted by adding 5mM $LaCl_3$ and 0.05 N HCl, and then the respective total ion quantities were quantified by determining the absorbance of Na^+, Ca^{2+} and K^+ by an atomic absorption spectrometer (AA-670, Shimadzu Seisakusyo, Kyoto, Japan). In the control group, cardiac perfusion was performed with Krebs-Henseleit buffer solution alone.

3. Measurement items and Statistical analysis

The absorbances of Na^+, Ca^{2+} and K^+ were determined, and the data were analyzed by the paired Student t test and the Turkey-Kramer method for intra-group and inter-group significance, respectively. The significance level was set to $P<0.05$.

Results

A. Experiment 1: Effects of BUP formulations on reperfusion-induced arrhythmias

1. Coronary flow (Fig. 2)

In the group treated with 5.0μg/ml LBUP, BUP or DBUP, coronary flow significantly decreased compared to the control group, showing a dose-dependent decreasing trend. However, there were no significant differences among the BUP formulations. Under ischemic conditions, coronary flow significantly decreased in all groups including the control group,

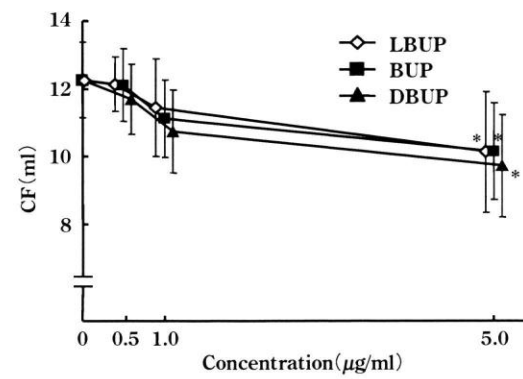

Figure 2 Effects of drug administration after 10 minutes on coronary flow

Values are means±SD. CF, coronary flow; LBUP, levobupivacaine; BUP, bupivacaine; DBUP, dexbupivacaine. *$P<0.05$, compared with control.

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and was increased by reperfusion to the baseline level that was observed before the ischemic condition.

2. HR (Fig. 3)

In **Fig. 3**, the data of HR that were measured 10 minutes after the treatment with the BUP formulations are shown. Compared to the control, a significant and dose-dependent decrease in HR was observed in all groups treated with the BUP formulations. Also, there were significant differences in the decrease of HR among the BUP formulations, showing an increasing trend of HR-reduction in the order LBUP<BUP<DBUP.

3. Incidence of sustained VF, VF, VT and PVC, and duration of VF and VT (Table 1, 2)

The incidence of sustained VF was 83% in the control, whereas it was significantly decreased to 17% by treating with LBUP at a concentration of 0.5μg/ml, and further to 0% at 1.0μg/ml or more. However, sustained VF did not occur by treating with BUP or DBUP over 0.5μg/ml, and there were no significant differences among the BUP formulations.

The incidence of VF was 100% in the control, whereas it was decreased to 50, 17 and 0% by treating with LBUP, BUP and DBUP of 0.5μg/ml, respectively; 1.0μg/ml, it was decreased to 8, 0 and 0%, respectively. No VF was observed in BUP formulations of 5.0μg/ml, showing a significant suppression of VF at all concentrations of each BUP formulations. In addition, an increasing trend of reduction in the incidence rate of VF was observed in the order LBUP< BUP<DBUP.

A similar trend was also observed for the incidence rates of VT and PVC, and durations of VF and VT.

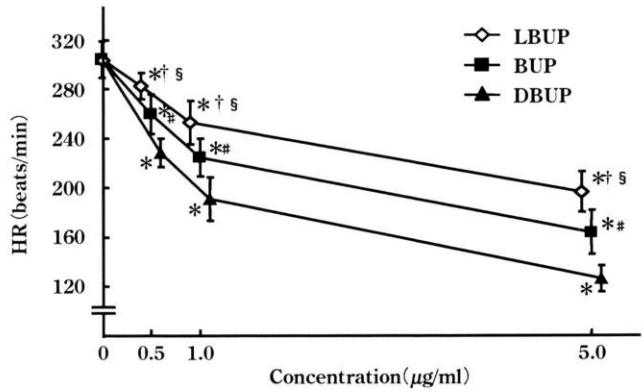

Figure 3 Effects of drug administration after 10 minutes on heart rate
There were significant differences between each group. Values are means±SD.
HR, heart rate; LBUP, levobupivacaine; BUP, bupivacaine; DBUP, dexbupivacaine.
*$P<0.05$, compared with control. †$P<0.05$, compared with BUP.
§$P<0.05$, compared with DBUP. #$P<0.05$, compared with DBUP.

Table 1 Effects of drug administration on the incidence of VF, sustained VF, and the duration of VF

Concentration	Incidence of VF (%)			Sustained VF (%)			Duration of VF (sec)		
(μg/ml)	LBUP	BUP	DBUP	LBUP	BUP	DBUP	LBUP	BUP	DBUP
Control	100	100	100	83	83	83	157±54	157±54	157±54
0.5	50*	17*	0*	17*	0*	0*	17±52*	1±2*	0*
1.0	8*	0*	0*	0*	0*	0*	3±10*	0*†	0*†
5.0	0*	0*	0*	0*	0*	0*	0*	0*	0*

(n=12)

Values are % or means±SD.
VF, ventricular fibrillation; LBUP, levobupivacaine; BUP, bupivacaine; DBUP, dexbupivacaine.
*$P<0.05$, compared with control. †$P<0.05$, compared with LBUP 1.0μg/ml.

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Table 2 Variables of reperfusion-induced ventricular arrhythmias

Concentration (μg/ml)	Incidence of VT (%)			Duration of VT (sec)			Incidence of PVC (%)		
	LBUP	BUP	DBUP	LBUP	BUP	DBUP	LBUP	BUP	DBUP
Control	100	100	100	9.4±4.6	9.4±4.6	9.4±4.6	100	100	100
0.5	75	50*	33*	4.1±3.9*	1.5±2.2*	1.5±1.8*	92	67*	58*
1.0	17*	0*	0*	0.8±2.2*	0*	0*	25*	17*	0*
5.0	0*	0*	0*	0*	0*	0*	0*	0*	0*

Values are % or mean±SD. (n=12)

VT, ventricular tachycardia; PVC, premature ventricular contraction; LBUP, levobupivacaine; BUP, bupivacaine; DBUP, dexbupivacaine. *$P<0.05$, compared with control.

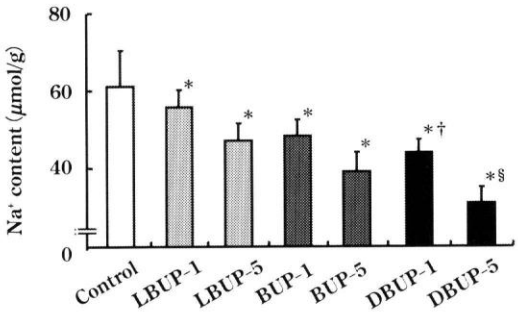

Figure 4 Effects of drug administration after 10 minutes on tissue Na^+ content.

Values are means±SD.

LBUP-1/5, levobupivacaine 1.0μg/ml/5.0μg/ml; BUP-1/5, bupivacaine 1.0μg/ml/5.0μg/ml; DBUP-1/5, dexbupivacaine 1.0μg/ml/5.0μg/ml.

*$P<0.05$, compared with control. †$P<0.05$, compared with LBUP-1. §$P<0.05$, compared with LBUP-5.

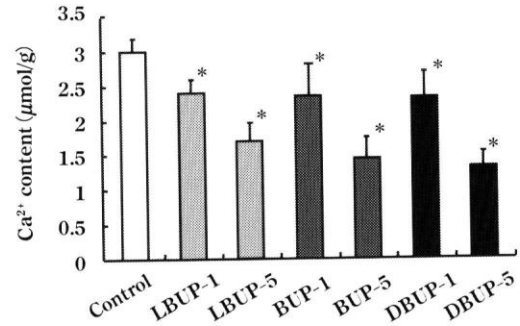

Figure 5 Effects of drug administration after 10 minutes on tissue Ca^{2+} content.

Values are means±SD.

LBUP-1/5, levobupivacaine 1.0μg/ml/5.0μg/ml; BUP-1/5, bupivacaine 1.0μg/ml/5.0μg/ml; DBUP-1/5, dexbupivacaine 1.0μg/ml/5.0μg/ml.

*$P<0.05$, compared with control.

B. Experiment 2: Effects of BUP formulations on the total tissue contents of Na^+, Ca^{2+} and K^+

1. Total Na^+ content (Fig 4)

A significant and dose-dependent decrease in total tissue Na^+ content was observed in all groups treated with the BUP formulations compared to that observed before the treatment with the BUP formulations. Furthermore, there was a decreasing trend in the total tissue Na^+ content in the order LBUP>BUP>DBUP.

2. Total Ca^{2+} content (Fig 5)

Although a significant and dose-dependent decrease in the total tissue Ca^{2+} content was observed in all groups treated with the BUP formulations compared to the control, there were no significant differences among the BUP formulations.

3. Total K^+ content (Fig. 6)

A significant decrease in the total tissue K^+ content was observed in the groups treated with any of the three BUP formulations, except the group treated with 1.0μg/ml LBUP, compared to the control. Each

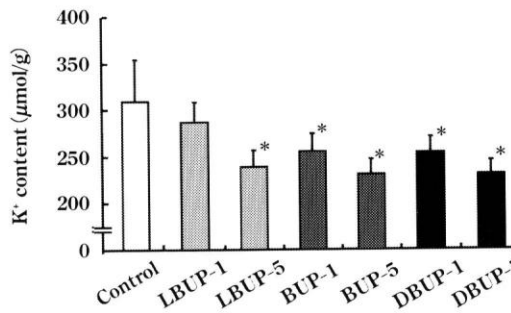

Figure 6 Effects of drug administration after 10 minutes on tissue K^+ content

Values are means±SD.

LBUP-1/5, levobupivacaine 1.0μg/ml/5.0μg/ml; BUP-1/5, bupivacaine 1.0μg/ml/5.0μg/ml; DBUP-1/5, dexbupivacaine 1.0μg/ml/5.0μg/ml.

*$P<0.05$, compared with control.

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of the three BUP formulations showed a dose-dependent decreasing trend in tissue K^+ content, but no significant differences were observed among the BUP formulations.

Discussion

Reperfusion damage in the heart denotes the phenomenon that cellular damage occurs after marked reoxygenation of myocardial cells that have been under a hypoxic condition; and reperfusion-induced arrhythmia is known to be an example of these. Although many possibilities have been speculated as factors in the occurrence of reperfusion-induced arrhythmias, currently, Ca^{2+} overload and oxygen free radical have been considered to be greatly involved[10,11]. However, BUP, an amide type, long-acting local anesthetic drug, is known to exert an antiarrhythmic effect via blocking Na^+ and Ca^{2+} channels[5]. Also, as reported by Tanz, et al[12], because BUP is long-acting and has high potency compared to lidocaine, it has been considered strongly cardiotoxic due to its strong Na^+ channel-blocking potency. In addition, it has been reported that BUP exerts pharmacological actions in a stereospecific manner and that LBUP with an S(−)-configuration exerts a lower cardiotoxicity compared to that of DBUP with a R(+)-configuration[1,2,5]. Thus, in the present study, the effects of BUP formulations on reperfusion-induced arrhythmias were examined, focusing attention on antiarrhythmic action and on stereospecificity in cardiotoxicity.

Under ischemic conditions, a consecutive series of biochemical events occurs, including ATP decrease, activation of ATP-dependent K^+ channel, extracellular K^+ efflux, and cell membrane depolarization, resulting in disorders of cellular conductivity or abnormalities of cell automaticity. In addition, with the decrease in ATP, intracellular H^+ concentration increases, and the Na^+/H^+-exchange system in the cell membrane is activated, which stimulates cellular H^+ efflux and Na^+ entry. Furthermore, it has been reported that Na^+/K^+-ATPase is inhibited by ATP deficiency, while the Na^+/Ca^{2+}-exchange system is stimulated, which results in drastic elevation of intracellular Ca^{2+} concentration[10,13]. In the present study, BUP formulations showed a dose-dependent suppressive effect on reperfusion-induced arrhythmias in the increasing order LBUP<BUP<DBUP, along with a similar suppressive effect of reducing HR in the same manner. As a reason for this, the following may be considered: LBUP has a lower affinity and a shorter duration of binding to Na^+ channels compared to those of BUP or DBUP; it therefore has lower potency of inotropic and chronotropic actions, showing faster recovery from the channel blocking[14]. Ropivacaine, like LBUP, has a S(−)-configuration, and shows a high binding selectivity to nerve membrane Na^+ channels, whereas it shows a low affinity to myocardial Na^+ channels. In contrast, BUP has a higher lipophilicity, and therefore exerts a stronger action and is long-acting, not only on nerve membrane Na^+ channels, but also on myocardial Na^+ channels[15]. In view of the cardiotoxicity, K^+ ions also play an important role. It has been reported that both ATP-dependent K^+ channel openers and blockers are effective in suppressing reperfusion-induced arrhythmias, and that K^+ channel openers exert cytoprotective effects[16,17]. Therefore, it can be speculated that some differences in the K^+-related effects may be involved in the HR-reducing effects of the BUP formulations. As described above, it is suggested that the differences in suppressive effect on reperfusion-induced arrhythmias, and those in HR-reducing effect among the BUP formulations, may be explained in terms of the differences in the mode of action on various ion channels or exchange systems.

The critical concentration of BUP to exert cardiotoxicity has been reported to be 4.0 to 5.0μg/ml[4,18], and that of LBUP and DBUP is considered to be almost the same. In fact, in the present study, HR was strongly suppressed by all of the three BUP formulations at 5.0μg/ml. Although the results of the present study were obtained in an *in vitro* system using the Langendorff method, and further investigations are required for clinical application, it was considered

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that a concentration of 1.0μg/ml, at which the incidence of sustained VF and VF could be suppressed, and LBUP, which exerted a lower HR-reducing effect, might be effective in suppressing reperfusion-induced arrhythmias.

In the present study, to investigate the different mode of action of the BUP formulations in **Experiment 1**, the total tissue contents of Na^+, Ca^{2+} and K^+ were measured 10 minutes after the administration of the BUP formulations. Total Na^+ content decreased, which suggested that the tissue concentration of Na^+ decreased, and that the Na^+ entry from extracellular matrix might be suppressed. In addition, the decrease in total Na^+ content in the order LBUP>BUP>DBUP was considered to reflect the differences in the potency of cardiac Na^+ channel-blocking activity, as observed in **Experiment 1**, which might affect the conduction system or action potential, and might be related to the differences in suppressive effects on reperfusion-induced arrhythmias and HR. According to the studies by Zapata-Sudo, et al[14], using the patch clamp method, there were no differences in the blocking actions on the L-type Ca^{2+} channels among the BUP formulations. In the present study, it was considered that the BUP formulations might exert Ca^{2+} overload-suppressive effect, because the total Ca^{2+} content decreased, but there were no significant differences among the BUP formulations. Therefore, some factors other than the Ca^{2+} channel may be involved in the different modes of action of the BUP formulations. As for the roles of K^+ channels in the electroactivities of the heart, many possibilities have been proposed, including maintenance of resting potential, determinations of the duration of action potential and the absolute refractory phase, build-up of automaticity, modification of myocardial electroactivitites by autonomic nervous activities, and adjustment of myocardial electroactivities under a condition of metabolic disorder, etc[13,19,20]. In addition, various kinds of myocardial K^+ channels and exchange systems have been identified[20]. Effects of BUP formulations on K^+ channels and exchange systems of K^+ have been reported in recent years, showing that BUP formulations could block HERG-channels and prolong QT[21]. Therefore, it is not necessarily appropriate to suggest the role of K^+ channels from the results of the present study, because of the presence of diverse channels. Nevertheless, it is considered that some differences in K^+ channel opening and/or blocking action may exist among the BUP formulations, in view of the decrease in total tissue K^+ content and the different mode of action on myocardial protection.

It is suggested that there may be differences in Na^+ channel-blocking action, which affects conduction systems and action potentials, among LBUP, BUP and DBUP, and that the BUP formulations may exert some different effects on K^+ channels. However, with regard to the effects on K^+ channels, more detailed investigations are considered necessary.

Conclusion

In the experiments on cardiac perfusion using BUP formulations, significant differences were observed, particularly in the effect on HR, showing HR-reducing effects in an increasing order LBUP<BUP<DBUP. In the experiments on the total tissue ion-content by atomic absorption analysis, significant differences were observed, particularly in Na^+ content, showing increases in total tissue Na^+ content in a decreasing order LBUP>BUP>DBUP.

Acknowledgments

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