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IN VIVO AND *IN VITRO* BRADYCARDIA INDUCED BY LOCAL ANESTHETICS IS POTENTIATED BY CALCIUM CHANNEL BLOCKERS

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Abstract : The present study examined the interactions of local anesthetics (LA) and calcium channel blockers (CCBs) on rhythmicity of heart using *in vivo* and *in vitro* experiments. ECG recordings were made from the anesthetized rats for *in vivo* preparations and spontaneously beating isolated rat right atrial potential for the *in vitro* experiments. The *in vivo* experiments with LA showed dose-dependent bradycardia with lignocaine (LIG, 100-500 µg/kg) and bupivacaine (BUP, 10-100 µg/kg). BUP was 4-5 times more potent than LIG. Verapamil (VML) and diltiazem (DTZ), CCBs also produced dose (10-100 µg/kg) -dependent bradycardia. However, none of them affected the PR/QT interval or QRS complex. Further, LA-induced bradycardia was potentiated by CCBs. In addition, flattening of P-wave in ECG was observed with doses (10-25 µg/kg) of LA in the presence of CCBs. Similarly, the *in vitro* experiments demonstrated a concentration-dependent decrease in atrial rate by BUP or VML. The BUP-induced decrease was potentiated in the presence of VML. Thus, the results suggest that CCBs potentiate the LA-induced bradycardia by involving pacemaker activity. Further, the flattening of P-wave in ECG serves as an early indicator of the cardiotoxicity produced by these drugs.

Key words : local anesthetic calcium channel blockers bradycardia bupivacaine lignocaine verapamil diltiazem

INTRODUCTION

Calcium channel blockers (CCBs) and local anesthetics (LA) are commonly used in clinical practice. CCBs are one of the first lines of therapy in hypertensive patients. In addition, LA are also used regularly for major and minor surgical procedures. Further, systemic administration of either of these agents is known to depress cardiac functions. The available *in vivo* experimental studies using LA and CCBs at toxic doses showed severe arrhythmia and death in experimental animals (1–3). Cardiac muscle

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contractions were also decreased in isolated paced ventricles by these drugs (4, 5). However, the effects on chronotropic properties of heart are not clear. Moreover, at toxic doses CCBs potentiate the cardio depressive action of LA (3, 6, 7). There is every chance for systemic absorption of LA, even in low concentration during surgery. Thus, the interactions between LA and CCBs assume great importance to the patients receiving CCBs. Further, no predictable indicators for such drug interactions and toxicity are available. Therefore, the present studies were undertaken to understand the effects of lower doses of LA (lignocaine-LIG and bupivacaine-BUP) and CCBs (verapamil-VML and diltiazem-DTZ) either alone or in combinations on the cardiac rhythmicity in anesthetized rats. Further, the *in vitro* interactions were also assessed on spontaneously beating rat right atrial preparations.

MATERIALS AND METHODS

All the experiments were performed according to the guidelines of the Institute of Medical Sciences, Banaras Hindu University, Varanasi for conducting the animal experiments. Care was taken to restrict the number of animals to the minimum possible.

Animal selection and groups

Adult albino rats (Charles Foster strain) of either sex weighing around 250 g were used in the present investigation. They were reared for a week in the departmental animal room and fed with rat feed (Hindustan Lever, India) *ad libitum* and had free access to water. Rats were randomly divided into 3 groups. Group1 and 2 were used for *in vivo* studies (ECG recording). Group 1 was further divided into four subgroups (7–11 rats in each group) to determine the dose response of LIG, BUP, VML and DTZ alone. Group 2 was also subdivided into four groups (7–9 rats in each group) to observe the effect of VML + LIG, DTZ + LIG, VML + BUP and DTZ + BUP. Group 3 was subjected to *in vitro* studies with right atrial preparations. This group was subdivided into 3 groups (4 rats in each group) to observe the effect of BUP/VML alone and BUP after pretreatment with VML.

In vivo experiments

Preparation of the animal and ECG recording

Overnight fasted rats were anaesthetized by intra peritoneal injection of urethane (1.5 g/kg body weight) and placed on a wooden dissection board. After tracheostomy, right jugular vein was carefully dissected out and cannulated with a fine catheter filled with normal saline. The catheter was used for injecting various drugs and normal saline. ECG was recorded with the help of fine needle electrodes connected in Lead II configuration and the potentials were recorded on a chart recorder (Physiograph, Bio-devices, India). After allowing a stabilization period of 30 min, initial recording was made for 2 min. Then 0.1 ml of normal saline/drug was injected and the recording was continued for initial 2 minutes and then for two min at every 5 min intervals up to 30 min. Heart rate was calculated from average R-R intervals recorded from 2 min after infusion of normal saline or drugs.

Experimental protocol

The dose-response of LIG (100–500 µg/kg); BUP, VML and DTZ (10–100 µg/kg) on heart rate and P-wave were determined. In addition, doses that produced arrhythmia were also considered. Further, the interaction of sub threshold doses of LA and CCB was also determined. In initial experiments it was observed that P-wave was abolished at very low dose of LA, CCBs and their combinations. Therefore, a separate set of experiments was carried out with LA, CCBs and their combinations using four rats in each drug/combination to find out the minimum dose required to flatten the P-wave.

In vitro recording of spontaneous atrial potentials

Animals and dissection

The procedure for isolation of atria and recording of the spontaneous atrial potential had been described earlier (8, 9). Briefly, the rats were killed by cervical dislocation and exsanguinations. The heart was carefully dissected out and placed in a Petri dish containing chilled Krebs-Ringer solution (4°C, pH=7.4) bubbled with 100% oxygen. The right atrium was carefully separated from the rest of the heart and fixed to a glass tube placed in an organ bath (volume 10 ml) containing oxygenated Krebs-Ringer maintained at 28 ± 1°C. The atrial tissue was placed within the loop of fine Ag-AgCl wire electrode. The atrial preparation was then allowed to equilibrate for 30 minutes before making the control recordings. The Krebs-Ringer solution was changed at every 15 minute intervals. The spontaneous potentials were recorded on the chart recorder after amplification.

Recording procedures

After stabilization period of 30 min control recordings were obtained for initial five min and then for 2 min at every five min intervals up to 30 min after exposure to individual drug. Atrial rate was calculated from initial 2 min. The effect of LA in presence of CCB was ascertained by pretreating the atria with VML for 5 min before the administration of BUP.

Drugs and solutions

The Krebs-Ringer solution had the following composition (mM): NaCl, 137; KCl, 2.68; $CaCl_2.2H_2O$, 1.8; $MgCl_2.6H_2O$, 0.88; $NaH_2PO_4.2H_2O$, 0.36; $NaHCO_3$, 7 and glucose 11.

Two drugs from local anesthetic category (lignocaine hydrochloride- LIG and bupivacaine hydrochloride- BUP) and two CCBs (verapamil hydrochloride- VML and diltiazem hydrochloride- DTZ) were taken to examine their effects on cardiac activity. Drugs were obtained from Sigma Chemical Company (USA). Stock solutions of the drugs were prepared in distilled water and further dilutions were made with Krebs-Ringer solution to make the desired concentration at the time of experiment.

Statistical analysis

All the values are presented as mean ± SEM. One way or two way analysis of variance (ANOVA) was used to examine

the differences between various groups. Student's t-test for paired/unpaired observations was applied to compare the two groups and is mentioned at appropriate places. A P value <0.05 was considered significant.

RESULTS

LA produced concentration-dependent decrease of heart rate

Local anesthetic LIG produced concentration-dependent decrease in heart rate. At a dose of 100 µg/kg, 4% decrease was seen and at 500 µg/kg there was 20% decrease. BUP (10–100 µg/kg) also produced dose-dependent decrease in heart rate with 3% and 14% decrease for 10 and 100 µg/kg, respectively. The dose-response curves of these two drugs are shown in Fig. 1A and are significantly different (P<0.05, 2 way ANOVA). The 50% of the maximal response produced at highest concentration used (IC_{50}) values of BUP was 40 µg/kg and for LIG it was 200 µg/kg.

Calcium channel antagonists produced concentration-dependent decrease in heart rate

Calcium channel antagonists DTZ and VML also showed dose-dependent decrease in heart rate. However, the decrease was similar (P>0.05, two way ANOVA) for both VML and DTZ at all concentrations (Fig. 1B). The decrease of heart rate was 2–3% at 10 µg/kg and it was nearly 20% at 100 µg/kg. Their IC_{50} values are nearly similar and IC_{50} for DTZ and VML were 25µg/kg and IC_{50} of VML 30µg/kg, respectively.

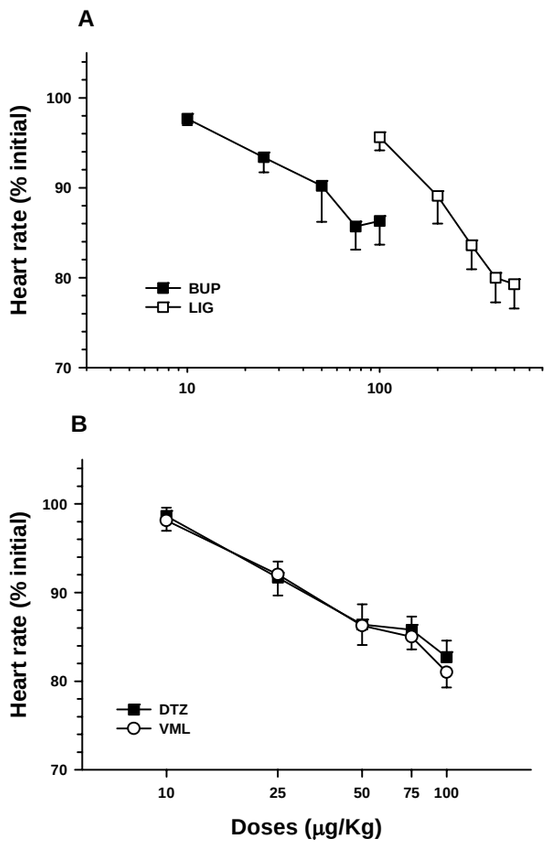

Fig. 1: Dose-response of local anesthetics (LA) and calcium channel blockers (CCBs) on heart rate. Dose-response of Bupivacaine (BUP, 10–100 µg/kg) and Lignocaine (LIG, 100–500 µg/kg) on heart rate of anesthetized rats in (A). The effect of different doses of Verapamil (VML) and Diltiazem (DTZ) in (B). Data are presented as mean±SEM from 6–11 experiments.

CCB pretreatment potentiated the bradycardia produced by LA

When LIG (100 µg/kg) or DTZ (10 µg/kg) administered alone the decrease in heart rate was only 2–5% but the combination of these drugs produced nearly 20% decrease in heart rate (Fig. 3). Similar change was also observed when LIG (200 µg/kg) was

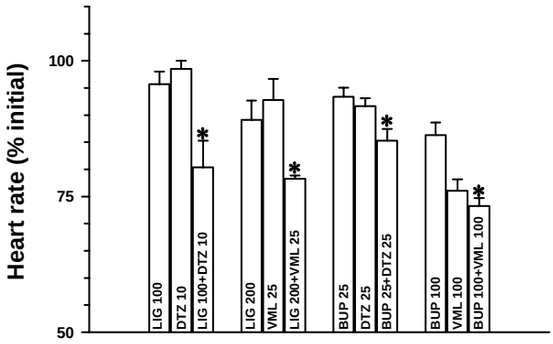

Fig. 2 : Combination of LA and CCBs produced significant potentiation of bradycardia in anesthetized rats. Lignocaine, LIG, n=11; Bupivacaine, BUP, n=7; Diltiazem, DTZ, n=10 and Verapamil, VML, n=7 alone and their combinations (VML+LIG, n=9; DTZ+LIG, n=8; VML+BUP, n=7, DTZ+BUP, n=9). The numbers inside the bar indicate doses of the drugs used. Data are presented as mean ± SEM. An asterisk (*) indicates P<0.05 (Students t-test for unpaired observations as compared to LA/CCB alone).

combined with VML (25 µg/kg). In case of BUP significant bradycardia was observed at 25 µg/kg with DTZ and 100 µg/kg with VML (P<0.05, Student's t-*tests* for paired observations) (Fig. 2).

LA/CCBs produced bradycardia and flattening of P-wave

R-R interval of ECG was prolonged after application of LA or CCBs. Other parameters like PR-interval, QT-interval and QRS complex remained unaltered. The most prominent change in ECG following low dose of these drugs was flattening of P-wave (Fig. 3). The Table I shows the minimum dose of different drugs (either alone or in combination) that produced flattening of P-wave in ECG without notable changes in QRS complex or QT interval. The required doses of drugs to flatten P-wave were very low when they were used in combination as compared to their individual doses (Table I).

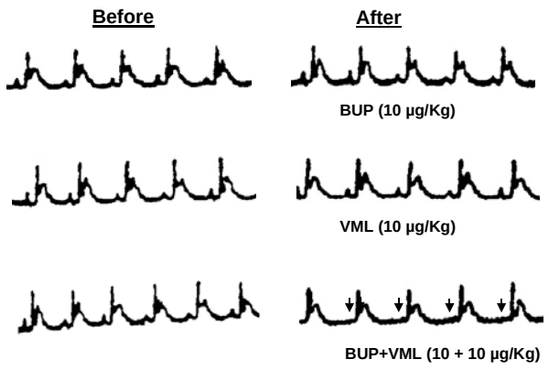

Fig. 3 : The original tracings of ECG before and after administration of Bupivacaine (BUP, 10µg/kg) and Verapamil (VML, 10µg/kg) and their combination (10+10 µg/kg). Please note the flattening of P-wave denoted by arrow (↓).

TABLE I : Minimum doses of different drugs (individual and in combination) at which P- wave was flattened (n = 4). LIG = Lignocaine, BUP = Bupivacaine, VML = Verapamil, DTZ = Diltiazem.

Drugs	*Doses (µg/Kg body weight)*
LIG	100
BUP	25
VML	25
DTZ	25
LIG + VML	25+10
LIG + DTZ	25+25
BUP + VML	10+10
BUP + DTZ	10+10

BUP, VML and their combinations decreased rate of spontaneously beating atria

A significant decrease of rate of spontaneously beating atria observed after combined application of BUP and VML (Fig. 4). On the individual administration, the decrease was 2–4% at the lowest concentration (0.3 µM) and 45–55% at the highest concentration (1.2 µM). When the

drugs were combined the decrease at 0.3 µM was 20% and at 1.2 µM it was 75%. The decline (75%) observed with the combined doses of BUP and VML in comparison to the individual administration was significant ($P<0.05$, Student's t-tests for unpaired observations, Fig. 4B).

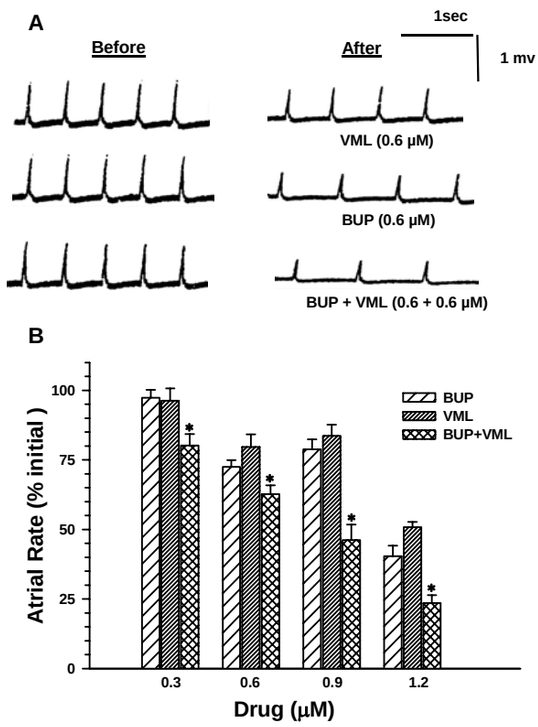

Fig. 4 : *In vitro* experiments showing the potentiation of LA and CCBs on atrial rate. Spontaneous potential recorded from isolated atria before and after Verapamil (VML, 0.6 µM) and Bupivacaine (BUP, 0.6 µM) alone and in combination are shown in (A). The horizontal line = 1 sec and the vertical line =1mv. Note a drastic decrease of atrial rate when these drugs were combined.

In (B) the mean±SEM data are shown in bar graphs before and after administration of Verapamil (VML, 0.3–1.2µM) and Bupivacaine (BUP, 0.3–1.2µM) alone and in combination. An asterisk (*) indicates $P<0.05$ as compared to BUP/VML alone.

DISCUSSION

The present results demonstrated that low doses of LA and CCBs alone can produce sinus bradycardia. Further, the effect of LA after pretreatment of CCBs was much greater. This effect was also evident in spontaneously beating isolated right atrial rate. Remarkable finding of this study was flattening of P-wave in ECG at very low doses of drugs when combined.

Previous experiments on dogs and mice documented the augmentation of negative chronotropic, inotropic, and arrhythmogenic effect of LA in presence of CCBs (3, 10, 11). These experiments were carried out using toxic doses. In contrast, the present investigation was performed using 20 times lesser dose used by others. Even with such low concentrations bradycardia and P-wave flattening was observed (Fig. 3). The combination of minimal doses of LA and CCBs produced greater bradycardia and P-wave flattening. Thus, it is possible that these agents produce synergistic action.

The *in vitro* data provide evidence regarding the action on the pacemaker activity. The *in vitro* experiments with isolated atria also confirmed the toxic effects of bupivacaine and verapamil combination. This indicated that the bradycardia observed were a direct effect on pacemaker cells and not a reflex phenomenon involving the central nervous system.

The comparative dose-response curve of lignocaine and bupivacaine (Fig. 1A) clearly demonstrated that potency of BUP was higher than LIG. The difference may be attributed to the fact that lignocaine affects

only the fast sodium channel (12), whereas, bupivacaine also blocks slow calcium channels (13, 14) and potassium channels (14).

Local anesthetics like lignocaine and bupivacaine are known to block fast sodium channel (12, 15) in pace maker cells. On the other hand calcium channel blockers (CCBs) verapamil and diltiazem selectively block L-type calcium channels and possibly also block fast sodium (16) and potassium channels (17). All these ionic currents contribute to the pacemaker potential/diastolic depolarization (18, 19). Thus, the local anesthetics and CCBs may influence the hyperpolarization activated current, I_f (20, 21) that determines spontaneous cardiac pace maker activity of sinoatrial node. The site of action of both the groups of drugs are being similar, it may be speculated that CCBs pretreatment will pronounce the negative chronotropic effect of local anesthetics. However, the exact ionic mechanisms for enhancement of cardiotoxicity by CCBs can not be ascertained from the present study. It is interesting to note that even less than one tenth of the arrhythmogenic dose of drugs could produce flattening of P-wave. Thus the present findings of severe bradycardia and flattening P-wave may be considered as an early feature for cardiotoxicity of LA and CCBs. In view of very frequent use CCBs in hypertensive patients, the chances of interaction between CCBs and LA are increasing. In the event of systemic absorption of very small amount of LA during regional anesthesia in minor surgical procedures the cardiac problems may be anticipated. Further, in such situation, the cardiac monitoring is required to detect the condition at early stage.

In conclusion, the present study documents that low dose of calcium channel blockers can enhance the local anesthetic induced negative chonotropic effect either *in vivo* or *in vitro* situations. These findings uncover the cardiotoxic effects of these drug interactions and also provide evidence for the early cardiotoxic sign.

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