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Influence of lidocaine on ouabain-induced inotropic response in rat atria

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Abstract

In this paper we demonstrated that lidocaine broadens the therapeutic range of ouabain action having a protective effect on ouabain-induced toxicity on rat atria. The lidocaine effect on therapeutic ouabain action was associated with the increase in the sensitivity of Na^+–K^+-ATPase related to a decreased in the equilibrium dissociation constant (K_d) of high affinity binding sites. Lidocaine suppressed the ouabain-induced tonotropic effect and arrhythmias, decreasing the number of low affinity binding sites (B_{max}) without changes in K_d. Blockade of Na^+–Ca^{2+} exchange with KB-R7943 or dual Na^+–Ca^{2+} channel with flunarizine, mimicked lidocaine effect increasing ouabain therapeutic action, extending its concentration range tolerated, delaying the onset of contracture. Lidocaine itself triggered negative inotropic response at high concentration. This effect was increased in the presence of flunarizine and verapamil but not by the inhibition of calcium/calmodulin with W-7. The mechanism underlying the lidocaine-induced negative inotropic response, appears to be different that underlying the positive inotropic effect on ouabain action. This study provides evidence that lidocaine can interact with the same or similar binding sites for ouabain in rat atrial tissue, providing a protective effect on ouabain-induced changes in contractility. The contribution of Na^+–Ca^{2+} exchange and/or Ca^{2+} overload on lidocaine effect is discussed.

Keywords: Lidocaine; Ouabain; Heart; Contractility; Binding assay

1. Introduction

Although each of a number of different drugs is able to prevent or suppress most arrhythmias, their use is limited by their narrow therapeutic index, that is, the narrow distance between effective and toxic concentrations. In addition, these drugs can cause toxicity at dosages presumed to be effective [1]. Furthermore, the electrical activity of the cardiac cells may be modified by changes in extracellular pH and ion concentration, as occur during ischemia. Electrical activity arises as a result of differences in ion (Na^+, Ca^{2+}, K^+) concentrations across the cell membrane caused by metabolism-depended processes [2].

Most antiarrhythmic agents block myocardial Na^+, Ca^{2+} or K^+ channels in a state-dependent manner. Lidocaine depresses membrane responsiveness in ventricular myocardial cells and in His-Purkinje system, altering the Na^+ channel predominantly by interacting with channel activation leading to channel opening [3]. In addition, the cardiodepressant effect of lidocaine is manifested by a decrease in heart rate and in tension [4,5] but has minimal effect on normal myocardial cells [2]. Lidocaine is advocated for the control of ventricular arrhythmias due to digitalis toxicity when these arrhythmias persist after cessation of digitalis intake and correction of hypokalemia [6,7].

However, lidocaine shares with other antiarrhythmogenic drugs the property of not only ameliorating but also triggering or exacerbating existing cardiac arrhythmias in their use as antiarrhythmic agents [8]. The cellular mechanisms of these actions is not fully understood yet. One biochemical entity for such actions is the electrogenic sodium pump and the monovalent cationic exchange mechanism of which is controlled by the Mg^{2+}-dependent ATP-hydrolytic function of the Na^+–K^+-ATPase [9]. Perturbation in the function of this pump is believed to be the underlying cause for digitalis toxicity [10].

The positive inotropic effect of cardiac glycosides invokes altered Na^+–Ca^{2+} exchange activity secondary to Na^+ pump inhibition with rise in intracellular Na^+ concentration. The rise in intracellular Na^+ concentration

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causes activation of sarcolemmal Na^+–Ca^{2+} exchange with intracellular Ca^{2+} overload. This in turn, induces oscillatory Ca^{2+} release from calcium stores associated with a transient ionic inward current. About 75% of the transient inward current is dependent on sarcolemmal Na^+–Ca^{2+} exchange while the remaining current is mediated by non-specific cation channels [11]. Both, therapeutic (positive inotropic effect) and toxic (tonic contracture and arrythmic effects) effects of ouabain are induced by oscillatory Ca^{2+} rise by transient inward current [11].

In this context, it has been reported that in embryonic mouse myocytes the Na^+–Ca^{2+} exchanger is absolutely required for the effect of cardiac glycosides on calcium [12]. Whatano *et al.* [13] suggested that inhibition of reverse mode Na^+–Ca^{2+} exchange or of Na^+–Ca^{2+} overload, reduced arrhythmogenic action of ouabain. They proposed that lidocaine produced a protective effect on ouabain-induced tonotropy and arrhythmias in isolated guinea pig atria; which might arise from its Na^+ channel blocking property. In fact, the inhibition of Na^+ channel may decrease oscillatory fluctuation in resting potential associated with the transient inward current [13]. However, it is interesting to differentiate between the lidocaine effects on therapeutic and toxic actions of ouabain and to determine if they arise from the same or different mechanism. Recently, it was reported that partial inhibition of Na^+–K^+-ATPase by non-toxic concentrations of ouabain causes hypertrophic growth and transcriptional regulation of several growth-related marker genes in neonatal rat cardiac myocytes by the activation of multiple signal transduction pathways [14].

Although, numerous studies have documented a low affinity of rat heart Na^+–K^+-ATPase for cardiac glycosides [15], it is also accepted that, despite the known reduced sensitivity of the rat myocardium towards digitalis [16]. Ouabain is able, under appropriate experimental conditions, to augment the contractile activity of the rat heart muscle [17]. Previous findings have suggested that alterations of the ionic environment and changes in the movements of ions through the plasma membrane could be alter the response to ouabain exhibited in rat cardiac muscle [18].

To further elucidate the interdependence between ouabain sensitivity and the cellular Na^+–Ca^{2+} exchange, the influence of lidocaine on the concentration-dependence of ouabain binding and on ouabain actions was studied in this paper. We observed that lidocaine-potentiated the inotropic positive effect (therapeutic action) with a decrease of tonic contracture (toxic action) of ouabain in isolated rat atria. Also, we offered a biochemical explanation of its actions as a product of the interaction with the same receptor sites involved in ouabain binding. Moreover, the protective effect of lidocaine against ouabain action in isolated rat atria was compared with selective inhibitors of Na^+–Ca^{2+} exchange. We also examined the action of ouabain and compared this effect with those of verapamil (calcium blocker), W-7 (calcium/calmodulin inhibitor), flunarizine (dual Na^+–Ca^{2+} channel blocker) and KB-R7943 (Na^+–Ca^{2+} exchanger blocker) on lidocaine-induced inotropic negative effect.

2. Material and methods

2.1. Animals

Adult male Wistar strain rats (250–300 g) were used. The animals, housed in standard environmental conditions, were fed with a commercial pelleted diet and water *ad libitum*. Experimental protocols were performed following the ''Guide to the Care and Use of Experimental Animals'' (DHEW Publication, NIH 80-23).

2.2. Atria preparation for contractility

Male Wistar rats were killed by decapitation. The left atria were carefully dissected from the ventricles, attached to a glass holder and immersed in a tissue bath containing Krebs–Ringer bicarbonate (KRB) solution gassed with 5% CO_2 in oxygen and maintained at pH 7.4 and at 30°. KRB solution was composed as described previously [19]. A preload tension of 750 mg was applied to the atria and tissues were allowed to equilibrate for 1 hr. The initial control values for contractile tension of the isolated atria were recorded by use of a force transducer coupled to ink writing oscillograph [20]. The preparations were paced with a bipolar electrode and an SK4 Grass Stimulator. The stimuli had duration of 2 ms and the voltage was 10% above threshold. Inotropic effects (dF/dt) were assessed by recording the maximum rate of isometric force development during electrical stimulation at a fixed frequency of 150 beats/min. Control values (=100%) refer to the dF/dt before the addition of drugs. The absolute value for dF/dt at the end of the equilibration period (60 min) was 7.8 ± 0.5 g/s. Cumulative dose–response curves were obtained according to the method of Van Rossum [21]. A maximal effect was achieved within 5 min after each dose.

2.3. Radioligand binding assay

Membranes were prepared as described previously [22]. In brief, atria were homogenized in an Ultraturrax at 4° in 6 vol. of potassium phosphate buffer, 1 mM $MgCl_2$, 0.25 M sucrose (buffer A) pH 7.5, supplemented with 0.1 mM phenylmethylsulphonylfluoride (PMSF), 1 mM EDTA, 5 μg/mL leupeptin, 1 μM bacitracin and 1 μM pepstatin A. The homogenate was centrifuged for 10 min at 3000 *g*, then at 10,000 *g* and 40,000 *g* at 4° for 15 and 90 min, respectively. The resulting pellets were resuspended in 50 mM phosphate buffer with the same protease inhibitor pH 7.5 (buffer B). Receptor ligand binding was performed as described previously by Noel and Godfraind [23]. The equilibrium binding of ^{3}H-ouabain

was measured after 10 min at 37° in 0.16 mL of incubation medium containing 0.2 mg protein with 5 nM ^{3}H-ouabain (specific activity: 23.2 Ci/mmol) and increasing concentrations of ouabain (10^{-8} up to 5×10^{-5} M). After incubation, samples were placed into 4 mL ice-cold buffer (0.25 M sucrose, 5 mM Tris–HCl, pH 7.4); and immediately filtered through GF/c glass fiber filters. The filters were rinsed with 8 mL of buffer, dried, placed in 10 mL Triton–toluene-based scintillation fluid and counted. The non-specific binding was estimated from samples incubated in the presence of 10^{-3} M unlabeled ouabain and accounted for 10–15% of total binding. Binding experiments were analyzed with Scatchard plots, which indicated the existence of high and low affinity sites. Curvilinear plots were analyzed using a weighted non-linear least square curve fitting program, LIGAND, assuming the existence of two independent classes of ouabain sites and making possible the calculation of dissociation constants (K_d) and number of binding sites (B_{max}).

2.4. Drugs

Ouabain, lidocaine and verapamil from Sigma Chemical Co.; flunarizine dihydrochloride from RBI; W-7 hydrochloride and KB-R7943 from Tocris. Stock solutions were freshly prepared in the corresponding buffers. The drugs were diluted in the bath to achieve the final concentration stated in the text.

2.5. Statistical analysis

Student's *t* test for unpaired values was used to determine the levels of significance. When multiple comparisons were necessary, after ANOVA, the Student–Newman–Keuls test was applied. Differences between means were considered significant if $P < 0.05$.

Table 1
Effect of different agents on basal contractility (d*F*/d*t*)

Drugs	d*F*/d*t* (g/s)	N
None	4.6 ± 0.4	8
Verapamil (1×10^{-9} M)	4.9 ± 0.5	7
Flunarizine (1×10^{-9} M)	4.4 ± 0.3	7
Lidocaine (1×10^{-6} M)	4.7 ± 0.5	8
W-7 (1×10^{-7} M)	4.9 ± 0.7	6
KB-R7943 (5×10^{-7} M)	4.3 ± 0.4	7

Values are mean ± SEM. N: the number of preparations tested.

3. Results

The ability of lidocaine to induce changes in d*F*/d*t* in rat atria is shown in Fig. 1. Lidocaine induced a decrease in atria contractility that was only observed at 1×10^{-4} M. Verapamil (L-type Ca^{2+} channel blocker, 1×10^{-9} M) but not W-7 (calcium/calmodulin blocker, 1×10^{-6} M) increased significantly the negative inotropic effect caused by lidocaine on atria contractility (Fig. 1a). Moreover, a dual Na^+–Ca^{2+} channel blocker flunarizine (1×10^{-9} M) and the selective Na^+–Ca^{2+} exchanger blocker KB-R7943 (5×10^{-7} M) also, increased significantly the inhibitory effect of lidocaine (Fig. 1a). Verapamil, W-7, flunarizine and KB-R7943 did not modify *per se* d*F*/d*t* of rat isolated atria at the concentration used (Table 1).

Because lidocaine might influence the cardiac Na^+–K^+-ATPase activity [24], we investigated if ouabain at different concentrations affected the contractile action of lidocaine, since the enzyme is a putative receptor of cardiac glycosides. As can be seen in Fig. 1b ouabain from 1×10^{-5} to 1×10^{-4} M induced an increase in the negative inotropic effect of lidocaine in a concentration-dependent manner; being 1×10^{-4} M ouabain, the more effective concentration to produce the maximal inhibition observed with

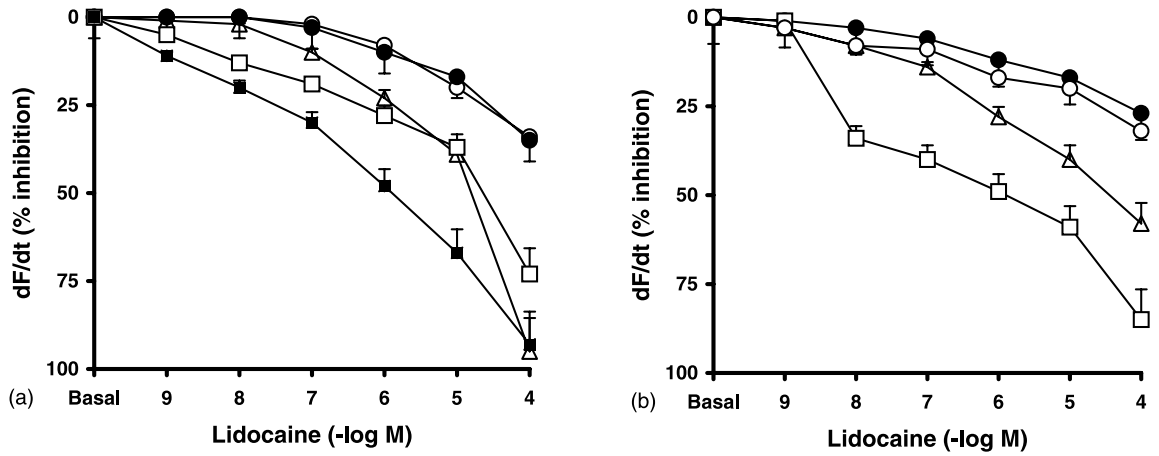

Fig. 1. (a) Effects of 1×10^{-9} M flunarizine (□), 5×10^{-7} M KB-R7943 (■), 1×10^{-9} M verapamil (△) and 1×10^{-6} M W-7 (○) on the dose–response curve of lidocaine (●) upon atria d*F*/d*t*. Tissues were incubated for 20 min in presence or absence of different inhibitors and then the dose–response curves to lidocaine were obtained. Values represent the mean ± SEM of seven experiments in each group. (b) Effects of different ouabain concentrations (1×10^{-6} M (○), 1×10^{-5} M (△) and 1×10^{-4} M (□)) on the dose–response curve of lidocaine (●) upon atria d*F*/d*t*. Tissues were incubated for 20 min in presence or absence of different inhibitors and then the dose–response curves to lidocaine were obtained. Values represent the mean ± SEM of seven experiments in each group.

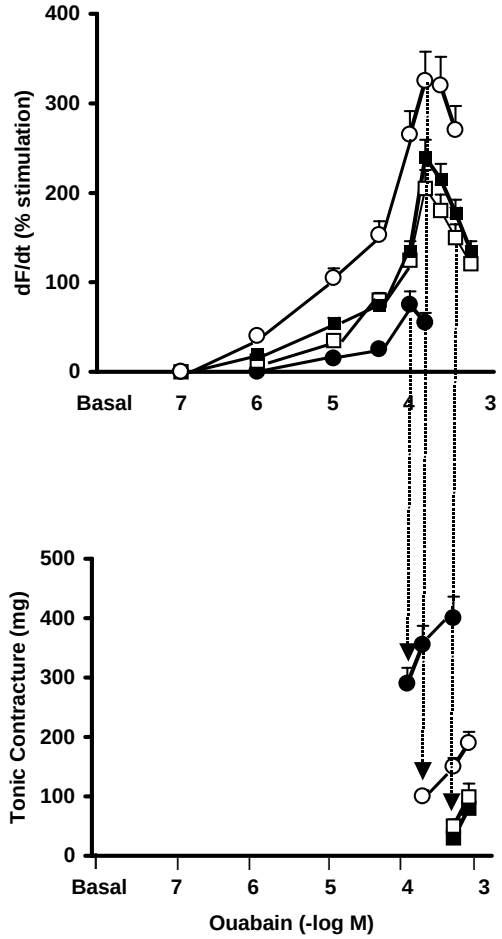

Fig. 2. Cumulative dose–response curve of positive inotropic (isometric tension) (upper panel) and toxic effect (tonic contracture) (lower panel) of ouabain on isolated rat atria. Each point represents the effect of ouabain on the parameters mentioned above after exposing the tissue for 30 min to the drug. Effect of 1×10^{-6} M lidocaine (○), 1×10^{-9} M flunarizine (□) and KB-R7943 (■) on the dose–response curve of ouabain (●). Points represent mean ± SEM of eight experiments in each group.

lidocaine. Ouabain trigger lidocaine maximal inhibition of d*F*/d*t*. On the contrary, ouabain at 1×10^{-6} M was uneffective in the study system.

On the other hand, Fig. 2 depicts the response of d*F*/d*t* (upper panel) and tonic contracture (lower panel) at different ouabain concentrations alone or in the presence of lidocaine, flunarizine and KB-R7943. Ouabain alone at low concentrations from 2.5×10^{-6} to 1×10^{-4} M increased d*F*/d*t*. At concentrations up to 2×10^{-4} M decreased d*F*/d*t* and contracture occurred. In the presence of lidocaine (1×10^{-6} M) or flunarizine (1×10^{-9} M) or KB-R7943 (5×10^{-7} M), added 20 min before ouabain, the inotropic positive effect of the cardiac glycoside on rat atria is shifted to the left enhancing d*F*/d*t*. On the contrary, all of these drugs at ouabain concentrations up to 2×10^{-4} M induced attenuation and delayed the onset in the capacity to trigger contracture (Fig. 2).

An original tracing showing the protective action of lidocaine on ouabain arrythmogenic action is shown in Fig. 3. At the concentration used lidocaine, flunarizine and KB-R7943 did not produced *per se* any influence on the d*F*/d*t* of atria before ouabain addition (Table 1).

Results obtained by measuring ^{3}H-ouabain binding sites in rat atria membranes, correlated with lidocaine-modified contractile activity of ouabain. As can be seen in Fig. 4, the equilibrium dissociation constant (K_d) of high affinity ^{3}H-ouabain binding decreased in cardiac membranes treated with lidocaine in comparison with untreated cardiac preparations. While the number of binding sites (B_{max}) remained unchanged in both treated and untreated membranes. On the contrary, lidocaine decreased the number of low affinity binding sites (B_{max}), while the K_d remained unchanged (Fig. 5). The non-specific binding in both treated and untreated lidocaine membranes, never exceeded the 15% of the total binding (untreated: 12 ± 1.5; lidocaine treated: 13 ± 1.8).

4. Discussion

The present study shows that lidocaine, a drug known to interact strongly with depolarized sodium channels, is capable to modify ouabain specific binding. Data also show that myocardium pretreated with lidocaine increased the range of ouabain therapeutic doses and diminished the toxic ones, exhibiting and enhancing in the therapeutic action and an attenuation in the toxic action of cardiac glycoside. This data provide evidence that the interaction between lidocaine and ouabain might be beneficial for ouabain action.

Our results indicate that lidocaine has different effects on the ouabain high and low affinity binding sites, i.e. on high affinity binding sites lidocaine decrease the K_d, behaving

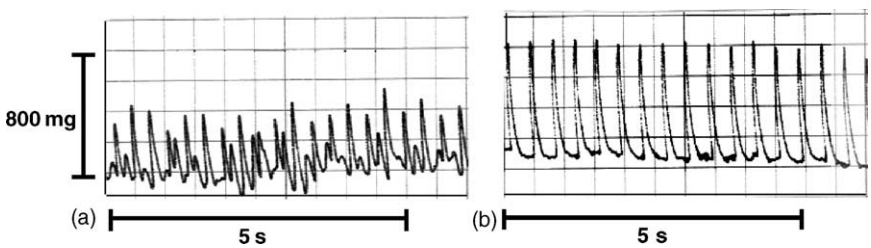

Fig. 3. Original tracing showing (a) arrythmogenic action of 2.3×10^{-4} M ouabain and (b) the antiarrythmogenic effect of 1×10^{-6} M lidocaine added 20 min before 2.3×10^{-4} M ouabain.

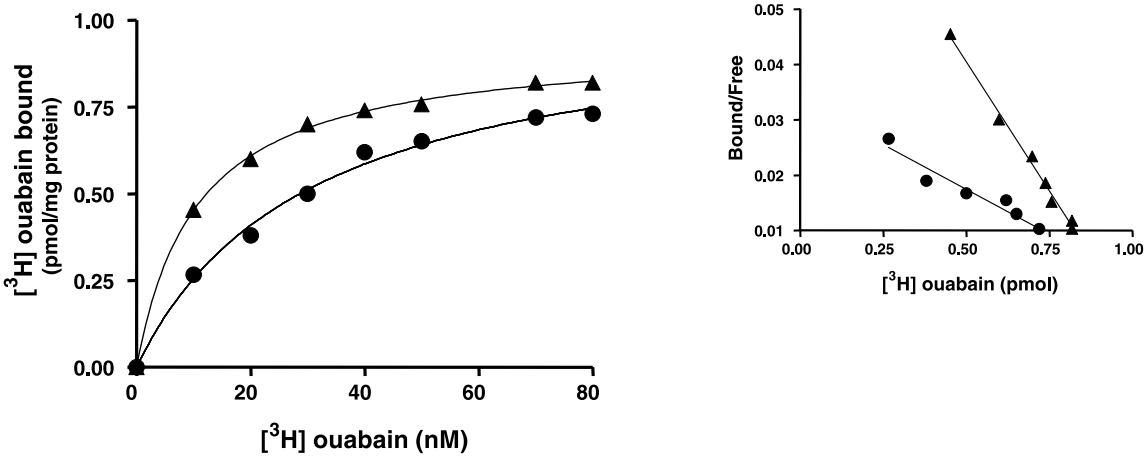

Fig. 4. Saturation assays and Scatchard analysis of high ^{3}H-ouabain binding sites to rat atria membranes from untreated (●) and 1×10^{-6} M lidocaine treated (▲) preparations. Binding parameters—ouabain alone: B_{max} (pmol/mg protein): 1.03 ± 0.05, K_d (nM): 30.5 ± 3.6; lidocaine + ouabain: B_{max} (pmol/mg protein): 0.95 ± 0.04, K_d (nM): 10.7 ± 0.5. These plots are mean ± SEM of representative of four other plots from experiments performed in duplicate.

as a competitive inhibitor while on low affinity binding sites lidocaine decrease B_{max}, behaving as a noncompetitive inhibitor. Studies on ouabain specific binding site in rat heart membrane have shown the presence of two ouabain binding sites with high and low affinity related to low doses-inotropic effect and high doses-tonic effect of the drug, respectively [23,25]. Our results appear to be consistent with this suggestion showing that lidocaine increased the positive inotropic effect of ouabain coincident with an increase to glycoside's affinity for high affinity binding site, while decreased in contracture would occur secondary to an decrease in the number of low affinity binding sites.

From these processes observed, it is expected that lidocaine not only acts blocking Na^+ channel but also it plays an essential role of plasma membrane Na^+–K^+-ATPase resulting in a rise in intracellular Na^+ levels as was described for most potent antiarrhythmic drugs in the treatment of digitalis-induce positive tonotropic effect [26]. The rise in intracellular Na^+ levels causes intracellular Ca^{2+} overload that in turn induces oscillatory calcium release from sarcoplasmic reticulum in resting potential [12] and increase contractility. Previously, is has been reported that lidocaine inhibits the Mg^{2+}-dependent ATP-hydrolytic function of the Na^+–K^+-ATPase in a similarly concentration-dependent fashion, exhibiting synergism with ouabain in this action [26–28]. As lidocaine have a role as the regulator of the monovalent cation trasmembrane transport, the cardiac Na^+–K^+-ATPase is a potential target for perturbations in the propagation of the cardiac action potential [29].

Several studies have demonstrated that the inotropic action and the arrhythmias induced by cardiac glycosides are embedded in their ability to inhibit myocardial Na^+–K^+-ATPase function [30–32]. At least three isoforms of the enzyme have been postulated to date (α_1, α_2 and α_3). The cardiac glycoside are thought to excert their effects by binding to both type sites (high and low affinity) of the

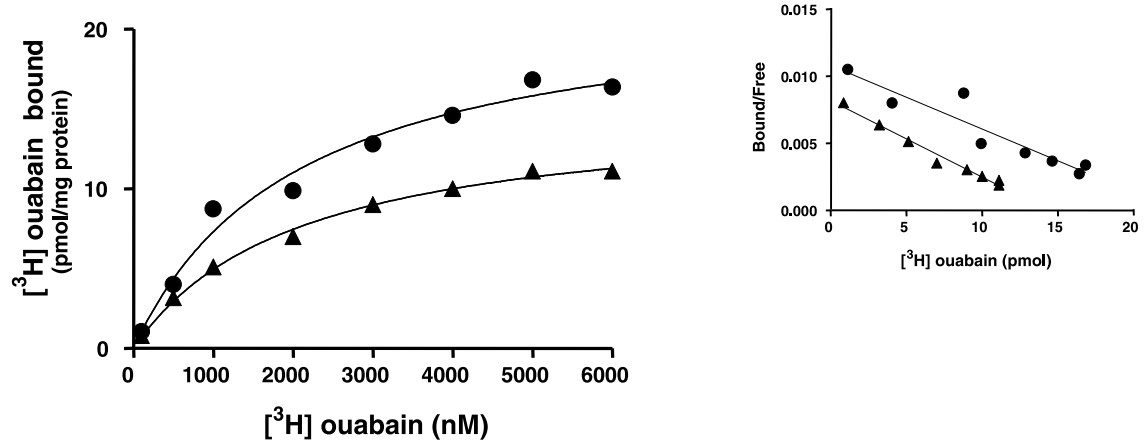

Fig. 5. Saturation assays and Scatchard analysis of low ^{3}H-ouabain binding sites to rat atria membranes from untreated (●) and 1×10^{-6} M lidocaine treated (▲) preparations. Binding parameters—ouabain alone: B_{max} (pmol/mg protein): 22.33 ± 1.8; K_d (nM): 2053 ± 244; lidocaine + ouabain: B_{max} (pmol/mg protein): 15.1 ± 0.6; K_d (nM): 2060 ± 207. These plots are mean ± SEM of representative of four other plots from experiments performed in duplicate.

different myocardial Na^+–K^+-ATPase isoforms [33,34]. The low affinity α_1 isoform being mainly responsible for positive inotropism while the α_2 and α_3 would be involved in the conduction of the action potential [35]. Low affinity binding sites of α_2 and α_3 Na^+–K^+-ATPase isoforms are probably relevant to cardiac glycoside to induce arrhythmias [35]. Similarly, it can be speculated that the same sites are responsible for the characteristic of lidocaine to modify the therapeutic and toxic action of ouabain. This property is probably related ultimately to alterations in calcium [36,37], the availability of which in the myocardium is the main component of cardiac force contraction. On the other hand, lidocaine significantly reduced the positive tonotropic effect of ouabain in isolated rat atria. This protective effect was also observed on lidocaine suppressed ouabain-induced arrhythmia [38] and indicated that lidocaine decreased the ouabain low affinity binding site to Na^+–K^+-ATPase in a concentration-dependent manner [26]. Borst *et al.* [39] demonstrated in beating ventricular strips from guinea pig heart, that lidocaine lowered ouabain binding by about one-third and extended the ouabain concentration range toleranted without toxicity by a factor of 3. The fact that flunarizine and KB-R7943 mimicked the lidocaine-modulated ouabain actions, agree this statement. These results are in agreement with Whatano *et al.* [13] that compared lidocaine with both Na^+–Ca^{2+} exchanger blocker (KB-R7943) and with Na^+–Ca^{2+} overload inhibitor (R-56865) showing a reduction of arrhythmogenic action of ouabain. Moreover, the specific action of flunarizine against ouabain-induced arrythmia was demonstrated [40]. It is important to note that flunarizine at concentration used was that *per se* no effect on basal atria dF/dt. This concentration is lower than the IC_{50} of this drug to block T-type calcium channel. So, we can include on flunarizine-induce modulation of ouabain actions the ability of the drug to interfere with the cellular Na^+ load [41]. The interdependence between digitalis sensitivity and the cellular Na^+ load was demonstrated in guinea pig ventricular strips [39].

Lidocaine strongly interacts with depolarized sodium channels and does not stabilize the sodium channel in an inactive state [3]. We report here that lidocaine itself has poor contractile action on myocardium. However, when ouabain bind to Na^+–K^+-ATPase revealed the inotropic negative effect of lidocaine upon isolated rat atria. The modulatory action of lidocaine on ouabain inotropic effect varied according with the concentration of the drugs used. Thus, from 1×10^{-7} to 1×10^{-6} M lidocaine potentiated ouabain increase dF/dt; but at 1×10^{-5} M lidocaine attenuated it and at 1×10^{-4} M lidocaine reversed the ouabain-positive inotropic effect to negative inotropic effect (data not show). These differences in the interdependence between the drugs could explain by the activation of multiple regulatory pathways, depending on the net influx of Ca^{2+} and the rise in intracellular Ca^{2+} concentration [14]. With several pharmacological interventions, such as treatment with Ca^{2+} channel and Na^+ channel inhibitors, we evaluated the central role of Ca^{2+} overload to prevent lidocaine-induced inhibition of atria dF/dt. Blockade of the Ca^{2+} entry with verapamil, Na^+–Ca^{2+} exchange with KB-R7943 and T-type Ca^{2+} channel with flunarizine but not calcium/calmodulin with W-7 were effective in significantly augmenting the negative effect of lidocaine on myocardium. However, the combined use of verapamil and lidocaine is complicated by a high incidence of bradychardia, which may limit the therapeutic usefulness of the former agent, as was also reported by others with ouabain [42,43]. The Na^+ and Ca^{2+} exchange inhibitors provides protection against Ca^{2+} overload by inhibiting the Na^+ overload, leading to a potentiation on the lidocaine negative inotropic effect. Indeed, the present results with flunarizine and KB-R7943 appear to be similar to those obtained with lidocaine at the level of Na^+ channels [44].

Finally, our results suggest that intracellular Na^+ and Ca^{2+} loading plays an important role in lidocaine and in the association of lidocaine/ouabain actions. Lidocaine at antiarrhythmic concentrations induced myocardium beneficial effects of ouabain-induced therapeutic action and protective effect on ouabain-induced toxic action and prevented the inotropic negative effect of lidocaine.

Acknowledgments

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