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Effect of lidocaine on α_1-adrenoceptors in cultured neonatal rat cardiocytes

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Abstract

The effect of lidocaine on α_1-adrenoceptors in cultured neonatal rat ventricular cardiocytes was studied by binding assay. When the cells were cultured in the presence of lidocaine, the binding of (±)-β-([^{125}I]iodo-4-hydroxyphenyl)-ethyl-aminomethyl-tetralone to the cells increased in a concentration- and time-dependent manner. The effect was due to an increase in maximum binding but not due to a change in the dissociation constant for the ligand. The increased number of α_1-adrenoceptors returned to the control level after culturing the cells without lidocaine. The stimulating effect on the number of α_1-adrenoceptors of lidocaine was also observed in the presence of norepinephrine. Other class I antiarrhythmic drugs such as procainamide, disopyramide, mexiletine and flecainide increased the number of α_1-adrenoceptors in the cells. These results suggest that cardiac responsiveness mediated by α_1-adrenoceptors is increased by class I antiarrhythmic drugs.

Keywords: Lidocaine; α_1-Adrenoceptor; Up-regulation; Cardiocyte; Culture

1. Introduction

The activity of the sympathetic nervous system and circulating catecholamines regulates myocardial function through α_1- as well as β-adrenoceptors. α_1-Adrenoceptors in the heart have a functional role distinct from that of β-adrenoceptors. The stimulation of α_1-adrenoceptors also leads to a variety of cellular responses including changes in contractility and/or excitability, metabolic alterations and cellular hypertrophy (Endoh, 1991; Terzic et al., 1993; Fedida et al., 1993). Stimulation of β-adrenoceptors produces positive inotropic and chronotropic actions which are mediated by Ca^{2+} channel phosphorylation (Trautwein et al., 1987) and alterations in gating properties via the adenylyl cyclase-protein kinase A pathway as well as directly through Gα subunit of G protein (Yatani et al., 1987).

Class I antiarrhythmic drugs such as lidocaine are clinically used in the treatment of ventricular arrhythmias. However, it is not known how the responsiveness of the heart changes during therapy. To see changes in the responsiveness of the heart produced by lidocaine, we investigated the change in α_1-adrenoceptors in cells elicited by long-term treatment with the drug, using cultured neonatal rat cardiocytes which beat spontaneously and which can be cultured for many days.

2. Materials and methods

2.1. Culture of neonatal rat cardiocytes

Cardiocytes were cultured as previously described (Mizuki et al., 1994). The ventricles of 2-day-old neonatal Wistar rats of either sex were minced into about 1-mm^3 pieces in Ca^{2+}- and Mg^{2+}-free phosphate-buffered saline (PBS(−)), and were treated with 2–5 ml of 0.12% trypsin and 0.03% collagenase in PBS(−) containing 10 mM Hepes, pH 7.4, for 4–5 min with mild agitation. The supernatant containing cardio-

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cytes was dropped into calf serum through a 100-μm nylon mesh. The digestion was repeated 7–8 times within 40 min, and the solution containing cardiocytes was made to 20% with respect to the calf serum concentration. After centrifugation of the solution at $700 \times g$ for 5 min, the resulting pellet was suspended in Dulbecco's modified Eagle's medium (DMEM) containing 10% fetal calf serum (HyClone, Logan, USA) and was plated on a Falcon culture dish (10 cm in diameter) for 90 min. The culture medium containing the cardiocytes was collected by decantation. More than 90% of the cells were cardiocytes by microscopic examination. The cells were cultured at 1.2×10^6 cells/dish (6 cm in diameter) at 37° C in 5% CO_2 in an incubator. The cardiocytes began to beat on the second day of culture. Medium was changed every day from the third day of culture. Lidocaine hydrochloride (Fujisawa, Osaka, Japan), disopyramide (Chugai, Tokyo, Japan), mexiletine (Nippon Boehringer Ingelheim, Kawanishi, Japan) or flecainide (Eisai, Tokyo, Japan) was added to the culture medium before the assay for the period indicated in the text. The α_1-adrenoceptors were measured after 5 days in culture. Most of the experiments were carried out using lidocaine as a representative of class I antiarrhythmic drugs.

2.2. Binding assays for α_1-adrenoceptors

The cultured cardiocytes were washed with 5 ml of PBS(−) 3 times and harvested with a rubber policeman in 50 mM Tris-HCl buffer, pH 7.5. The cells were homogenized with a Biotron (at level 9 for 10 s × 2) and were centrifuged at $20000 \times g$ for 20 min. The resulting pellet was homogenized in the same buffer and centrifuged again. The final pellet was suspended in the buffer and was used as a membrane fraction.

α_1-Adrenoceptors were measured using the radiolabeled α_1 antagonist, (±)-β-([^{125}I]iodo-4-hydroxyphenyl)-ethyl-aminomethyl-tetralone ([^{125}I]HEAT, specific activity 2200 Ci/mmol, NEN Research Products, Glossmann et al., 1981). The membrane fraction of the cultured cardiocytes was incubated in triplicate with various concentrations of [^{125}I]HEAT in 50 mM Tris-HCl buffer (pH 7.5) at 25° C for 60 min. [^{125}I]HEAT bound to the membrane was separated from free ligand by filtration through a GF/B glass fiber filter (Whatman), and the filter was washed four times with 5 ml of cold Tris-HCl buffer. The radioactivity of the filters was measured in a gamma counter with an efficiency of 85% (Aloka, Tokyo, Japan). The specific binding of [^{125}I]HEAT was defined as the total binding minus the non-specific binding, which was determined in the presence of 10 μM phentolamine.

The protein concentration was measured by the method of Lowry et al. (1951).

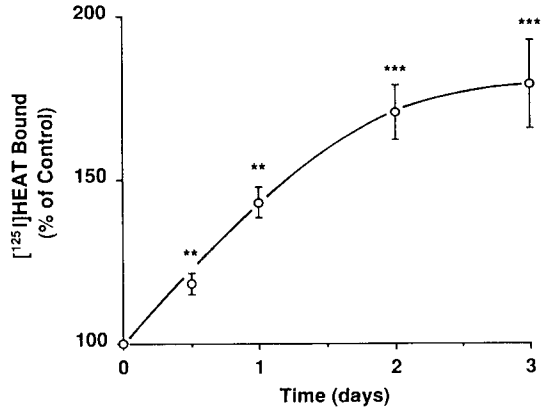

Fig. 1. Time course of increase in [^{125}I]HEAT binding to cultured cardiocytes by lidocaine. The cardiocytes were prepared from ventricles of 2-day-old rats by digestion with collagenase and trypsin, and were cultured for 5 days. Before the assay, the cells were cultured in the presence of 10^{-3} M of lidocaine for the time indicated on the horizontal axis. α_1-Adrenoceptors were measured by incubating the membrane fraction of the cells with 50 pM of [^{125}I]HEAT, as indicated in Materials and methods. Each value is expressed as a percentage of that of control cells which were cultured in the absence of lidocaine. Data are means ± S.E.M. of three to nine separate experiments. The control value was 85.9 ± 5.4 fmol/mg protein. ** $P < 0.01$, *** $P < 0.001$ significantly different from control.

Data are given as means ± S.E.M. Statistical difference was calculated by Student's t-test.

3. Results

3.1. Effect of lidocaine on α_1-adrenoceptors in cardiocytes

The binding of [^{125}I]HEAT, at the concentration of 50 pM, to the cardiocytes was increased by the addition of lidocaine to the culture medium. The effect was evident from 12 h after addition of lidocaine (10^{-3} M) and the binding was increased by 45%, 70% and by 80% over control after 1, 2 and 3 days of incubation with 10^{-3} M lidocaine, respectively (Fig. 1). The stimulating effect of lidocaine on binding was observed in a dose-dependent manner (Fig. 2). After 1 day of treatment, the binding to receptors tended to increase following incubation with 10^{-5} M of lidocaine and was significantly increased to 120 ± 5%, 135 ± 7% and 143 ± 5% of control by 10^{-4} M, 3×10^{-4} M and 10^{-3} M of lidocaine, respectively (clinical concentration of lidocaine in serum: 6×10^{-6}–3×10^{-5} M). Fig. 3 shows characteristics of the [^{125}I]HEAT binding to the cardiocytes treated with 10^{-3} M lidocaine for one day. At various concentrations of [^{125}I]HEAT, the binding was higher in the cardiocytes treated with lidocaine than in the control cardiocytes. Scatchard analysis of the data shows that the maximum binding (B_{max}) value for the

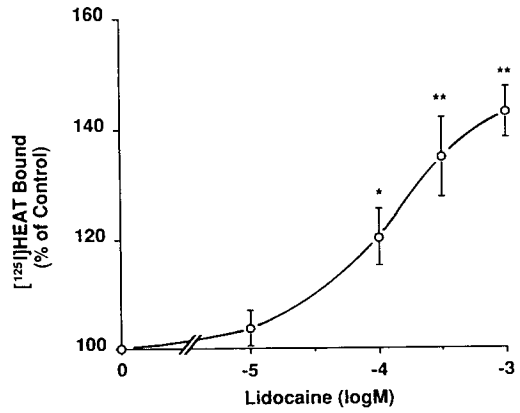

Fig. 2. Concentration dependence of the increase in [^{125}I]HEAT binding to cardiocytes by lidocaine. The cells were cultured for 1 day in the presence of various concentrations of lidocaine. Each value is expressed as a percentage of the control value measured at 50 pM [^{125}I]HEAT. Data are means ± S.E.M. of three to nine separate experiments. The control value was 82.9 ± 3.3 fmol/mg protein. * $P < 0.05$, ** $P < 0.01$ significantly different from control.

lidocaine-treated cells (190 ± 24 fmol/mg protein, n = 5) was 50% higher ($P < 0.05$) than that of control cells (126 ± 9 fmol/mg protein, $n = 5$) without a change in dissociation constant (K_d) value (35 ± 4 pM in control, 28 ± 5 pM in lidocaine-treated cells, Fig. 3). When lidocaine and other antiarrhythmic drugs were added to the incubation mixture of the binding assay, they did not increase binding to α_1-adrenoceptors (data not shown), instead 1 mM lidocaine inhibited [^{125}I]HEAT binding by more than 50%. Thus, the increase in α_1-adrenoceptor binding elicited by lidocaine is due to an increase in the number of receptors in the cells but not due to the direct interaction of antiarrhythmic drugs with α_1-adrenoceptors or the radioligand.

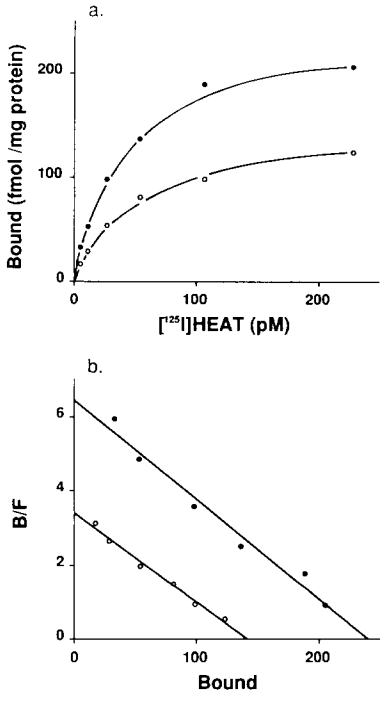

Fig. 3. Change in the characteristics of α_1-adrenoceptors in cardiocytes cultured in the presence of lidocaine. The cells were cultured in the presence (●) or absence (○) of 10^{-3} M of lidocaine for 1 day as Fig. 2. Specific binding of [^{125}I]HEAT to the membrane fraction (a) and its Scatchard plot (b) of a representative of five separate experiments are shown. Each value is the mean of triplicate determinations which varied less than 5%. B_{max} and K_d values: 142 fmol/mg protein and 42 pM in control; 238 fmol/mg protein and 37 pM in lidocaine-treated cells, respectively.

3.2. Reversibility of up-regulation of the α_1-adrenoceptors

The reversibility of the stimulating effect of lidocaine on α_1-adrenoceptors was examined. Treatment with 10^{-3} M of lidocaine for 24 h increased the [^{125}I]HEAT binding as described above. The increased α_1-adrenoceptor binding returned to 113 ± 3% of control level when the cells were cultured in the absence of lidocaine for another 24 h (data not shown in figure).

3.3. Interaction of lidocaine and norepinephrine on the increase in adrenoceptors

As lidocaine is often administered with catecholamines, the interaction of lidocaine with norepinephrine on α_1-adrenoceptors in cardiocytes was investigated (Fig. 4). Norepinephrine had little effect on the [^{125}I]HEAT binding to the cells. When lidocaine was added with norepinephrine, the [^{125}I]HEAT binding increased by 65% of that of the cells treated with norepinephrine alone.

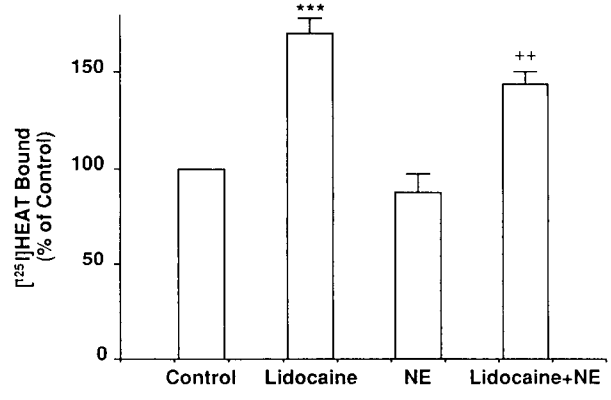

Fig. 4. Effect of norepinephrine on the increase in [^{125}I]HEAT binding to cardiocytes by lidocaine. The cells were cultured in the presence of *l*-norepinephrine (NE, 10^{-6} M) or lidocaine (Lidocaine, 10^{-3} M) or both for 2 days before assay. Each value is expressed as a percentage of the control value which was determined at 50 pM [^{125}I]HEAT. $n = 3$–6, *** $P < 0.001$ significantly different from the control, ++ $P < 0.01$ significantly different from cells cultured with norepinephrine alone.

Table 1
Effect of class I antiarrhythmic drugs on α_1-adrenoceptors of cardiocytes

Drugs	Class	[^{125}I]HEAT binding (fmol/mg protein)	Percentage of control
None		88.6± 6.9	100
Disopyramide	Ia	136.0±14.2 [b]	153
Lidocaine	Ib	113.6± 5.4 [b]	128
Mexiletine	Ib	134.7± 7.6 [c]	152
Flecainide	Ic	133.6±12.6 [a]	151

Cardiocytes were cultured with disopyramide (10^{-4} M), lidocaine (10^{-4} M), mexiletine (10^{-4} M) or flecainide (10^{-5} M) for 2 days and α_1-adrenoceptors were measured by binding assay using 50 pM of [^{125}I]HEAT. Each value is expressed as a percentage of that of control cells which were cultured in the absence of any drug. Data are means±S.E.M. of three separate experiments. [a] $P < 0.05$, [b] $P < 0.01$, [c] $P < 0.001$ significantly different from control.

3.4. Effect of class I antiarrhythmic drugs on α_1-adrenoceptors in cardiocytes

To see whether the increase in the number of α_1-adrenoceptors occurs only with lidocaine or whether this phenomenon occurs with other antiarrhythmic drugs, we investigated the effect of class Ia, Ib and Ic drugs on α_1-adrenoceptors. As shown in Table 1, disopyramide (Ia), lidocaine, mexiletine (Ib) and flecainide (Ic) significantly increased [^{125}I]HEAT binding to the cells. These results indicate that the increase in the number of α_1-adrenoceptors in cardiocytes is a phenomenon common to class I antiarrhythmic drugs.

4. Discussion

In the present study, we found that lidocaine increased [^{125}I]HEAT binding to cultured neonatal rat ventricular cardiocytes in a time- and dose-dependent manner. The increase in the binding was due to a change in the number of the receptors without a change in the affinity of the receptors for the ligand. In our previous report, we showed that the number of β-adrenoceptors and adenylyl cyclase activity stimulated by a β-agonist, isoproterenol, were increased when the cardiocytes were cultured in the presence of lidocaine (Mizuki et al., 1994). The increase in β-adrenoreceptors was also observed by culturing the cells with other class I antiarrhythmic drugs. Taken together, our results suggest that when lidocaine and other class I antiarrhythmic drugs are administered, the responsiveness of α_1- and β-adrenoceptors of the heart to catecholamine may increase.

One of the major problems of therapy with antiarrhythmic drugs is the proarrhythmic effect of the drug, that is, during therapy, paradoxical arrhythmia tends to occur frequently. The increase in α_1-adrenoceptors, in addition to the increase in β-adrenoceptors, may have a role in this proarrhythmic effect of the antiarrhythmic drugs, because physiological and pathological states of the heart, including cardiac contractility and rhythm, are regulated by α_1-adrenoceptors as well as by β-adrenoceptors. In addition, accumulating evidence suggests that α_1-adrenoceptor stimulation plays an important role in the occurrence of arrhythmia, in particular in conditions such as myocardial ischemia and reperfusion (Corr et al., 1989; Kurtz et al., 1991) and during halothane anesthesia (Maze et al., 1985). It is interesting to speculate that the increase in α_1-adrenoceptors produced by antiarrhythmic drugs found in this study may correlate with the proarrhythmic effect of the drugs.

It is not clear how stimulation of α_1-adrenoceptors leads to arrhythmia (Terzic et al., 1993). Stimulation of α_1-adrenoceptors leads to activation of a number of signal transduction pathways such as phosphatidylinositol metabolism, Ca^{2+} transients, L-type Ca^{2+} channels, delayed rectifier K^+ current, Na^+/H^+ exchange and Na^+,K^+-ATPase (Terzic et al., 1993). Among these pathways, activation of Na^+/H^+ exchange might be responsible for arrhythmia through an increase in intracellular Na^+ resulting in Ca^{2+} overload by the net uptake of Ca^{2+} via the Na^+/Ca^{2+} exchange (Terzic et al., 1993).

In the present study, the increased number of α_1-adrenoceptors elicited by lidocaine returned near to the control level after the cells had been cultured in the absence of lidocaine for 1 day. Thus, from the clinical point of view, it is suggested that the increased sensitivity to sympathomimetic amines of the hearts of patients treated with lidocaine lasts for several hours after cessation of lidocaine treatment.

In our unpublished experiments, when the cells were cultured with 10^{-6} M norepinephrine alone, the number of β-adrenoceptors was decreased significantly to 30% of the control. The decrease in β-adrenoceptors was also observed in isoproterenol-treated cultured chick cardiocytes (Marsh, 1989), while α_1-adrenoceptors had a tendency to decrease but the effect was not significant. This difference between the changes in α_1- and β-adrenoceptors elicited by agonists suggests that mechanisms for the regulation of the number of α_1- and β-adrenoceptors are different.

The stimulating effects of lidocaine on number of α_1-adrenoceptors in the cardiocytes were observed also in the cells cultured with norepinephrine. When the cardiocytes were treated with both norepinephrine (10^{-6} M) and lidocaine (10^{-3} M), beating was not inhibited (data not shown), although it was completely stopped in the presence of lidocaine (10^{-3} M) alone. The fact that α_1-adrenoceptors increased even in the beating cells cultured with norepinephrine and lidocaine suggests that it is not likely that the change in mechanical force, that is, the change in the beating of

the cells, is involved in the increase in the number of α_1-adrenoceptors.

Class I antiarrhythmic drugs other than lidocaine also increased the number of α_1-adrenoceptors in the cardiocytes. Thus, it is plausible that the inhibitory action on the sodium channel of the drugs caused alterations in ion movements in the cell and consequently intracellular events may be involved in this phenomenon. In conclusion, our data showed that the number of α_1-adrenoceptors in cultured cardiocytes was increased by treatment with lidocaine. It is suggested that an increase in the number of α_1- and β-adrenoceptors synergistically or additively augments the reactivity of the heart to sympathomimetic amines when class I antiarrhythmic drugs are administered.

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