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Halothane, Isoflurane and Enflurane Potentiate the Effect of Noradrenaline on Ventricular Automaticity in the Rat Heart: Evidence of the Involvement of Both α- and β-Adrenoceptors

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Abstract—Direct evidence has been sought as to what extent the sensitization of heart to the arrhythmogenic action of sympathomimetic drugs in the presence of the inhalatory anaesthetics, halothane, isoflurane and enflurane, is mediated by either α- or β- adrenoceptors. For this purpose, the effects of isoprenaline, noradrenaline and phenylephrine on ventricular automaticity induced by local injury have been studied in the isolated right ventricle of the rat. Isoprenaline was more potent in increasing ventricular automaticity than either phenylephrine or noradrenaline. The anaesthetic potentiated the effects of noradrenaline, as well as that of higher concentrations of phenylephrine, but not those of isoprenaline. These results support the contention that increases in ventricular automaticity induced by sympathomimetic drugs are mainly mediated by adrenoceptors of the β-type. However, the simultaneous activation of both α- and β-adrenoceptors seems to be necessary for the effect of the anaesthetics in sensitizing the heart to sympathomimetic drugs.

Inhalatory anaesthetics sensitize the heart to the arrhythmogenic potential of catecholamines (Katz & Epstein 1968). The mechanism by which arrhythmias are produced is not clear, but it has been mainly attributed to activation of adrenoceptors of the β-type (Szekeres & Papp 1980). Nevertheless, there is evidence indicating that α-adrenoceptors also mediate this effect. For example, the degree of protection induced by the selective α_1-adrenoceptor antagonist prazosin, against the arrhythmogenic effect of adrenaline in halothane-anaesthetized dogs was greater than that obtained after administration of the β-blocking drug metoprolol (Maze & Smith 1983). Other α-adrenoceptor blocking drugs, such as droperidol or doxazosin, have also been shown to protect against arrhythmogenic doses of adrenaline during halothane anaesthesia in dogs (Maze et al 1985). However, these reports supporting a role of α-adrenoceptors in arrhythmias induced by catecholamines acting with inhalation anaesthetics are mainly based on indirect findings related to the effectiveness of α-adrenoceptor blocking drugs on this type of cardiac dysrhythmia.

The aim of the present work was to determine the effects of inhalation anaesthetics on the enhancement of cardiac automaticity induced by catecholamines in the rat heart. We attempted to obtain direct evidence, using α- and β-adrenoceptor agonists, as to what extent the sensitization of heart to the arrhythmogenic action of catecholamines in presence of the anaesthetics is mediated by β-adrenoceptors, as well as the possible involvement of α-adrenoceptors in this effect. Halothane, isoflurane and enflurane were the anaesthetics and isoprenaline, phenylephrine and noradrenaline were the sympathomimetic amines chosen because of their agonistic activity at β-, α- and at both β-, and α- receptors, respectively (Weiner 1985). Ectopic cardiac automaticity was induced by local injury of the isolated right ventricle of the rat which produces a sustained abnormal rhythm (Hernández & Serrano 1982).

A brief account of part of the work has been reported (Laorden et al 1987).

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Materials and Methods

The right ventricle of Sprague-Dawley rats of either sex was isolated as described by Hernández & Serrano (1982). After the rats had been killed by a blow to the head, the heart was removed and placed in warm Tyrode solution. The right ventricle was freed, then the atrial end was damaged by crushing with Starling forceps, and the ventricle fixed to a metallic support, the apical end being attached to a Grass FT-03 force-displacement transducer by a nylon thread. We used a 30 mL organ bath with a porous plate at the bottom to ensure effective aeration, the Tyrode batheing solution contained (mM): NaCl 136·9; KCl 5·0; $MgCl_2$ 1·05; $CaCl_2$ 1·8; $NaPO_4H_2O$ 0·4; $NaCO_3H$ 11·9; dextrose 5·0. The solution was maintained at 37°C and bubbled with 95% O_2 and 5% CO_2.

Contractions were recorded on a Dynograph Beckman Polygraph. The resting tension was set at 1 g. The right ventricle was allowed to stabilize for at least 10 min. Only ventricles having a stable basal rate, with a variation of less than 10 beats min^{-1}, and contractile activity at the end of the stabilization period, were used.

Isoprenaline HCl, noradrenaline HCl (Sigma Chemical Co.) and phenylephrine HCl (Andreu Lab. Spain) were added to the organ bath in 0·1 mL of water. Drugs were tested in concentrations ranging from 10^{-11} to 10^{-5} M. Dose–response curves of the three amines were constructed both in the absence and presence of either halothane (ICI), isoflur-

ane or enflurane (Abbott) in a concentration of 0·1 v/v % which is devoid of any effect on this preparation. These anaesthetics were added to the O_2/CO_2 gas mixture bubbled through vaporizers and calibrated by using an Emma Engström multigas analyser. An additional period of 15 min was allowed to elapse in the presence of each anaesthetic, before construction of dose–response curves with the sympathomimetic agents, to obtain equilibration between the gas/liquid phase (Puig et al 1988). A drug's effect was defined as the maximal change in the automatic frequency of the preparation for 1 min following drug administration.

The results were expressed as percentages relative to the control frequency of the preparation. To compare the excitatory effect of the sympathomimetic drugs used in the absence and in the presence of the anaesthetics, we measured for each set of experiments the corresponding ED50, defined as the mean drug concentration that increased ectopic ventricular automaticity by 50%. In the analysis of differences between doses and between drugs, we used a two-way analysis of variance and then Student's *t*-test for individual comparisons. Also, we used an analysis of linear regression with the log of doses and the log of response for the estimation of ED50. A *P*-value less than 0·05 was considered to indicate statistical significance.

Results

The effects of isoprenaline, phenylephrine and noradrenaline

Fig. 1 shows typical responses to addition of the agents to a batheing solution containing a right ventricle with ectopic automaticity. As can be seen, each of these amines effectively increased the automatic activity of the preparation during the time they were present in the medium. After washout, the

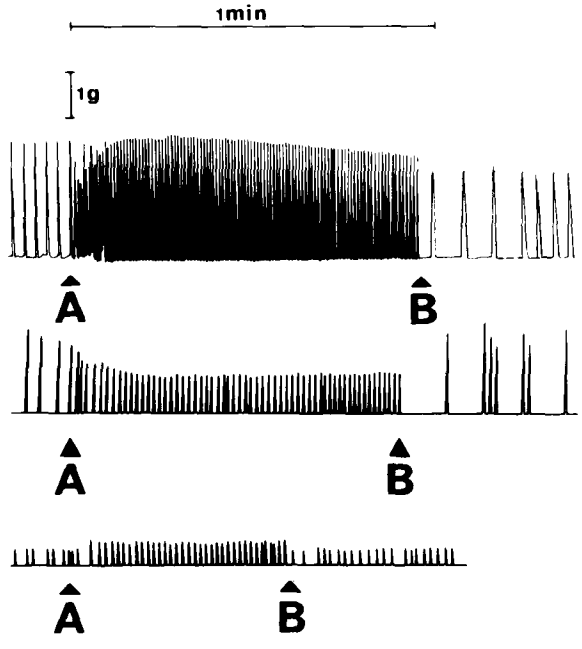

FIG. 1. Effects of isoprenaline (top), noradrenaline (centre) and phenylephrine (bottom) (10 μM applied at A) on ventricular automaticity induced by local injury. B represents the start of wash out of each drug.

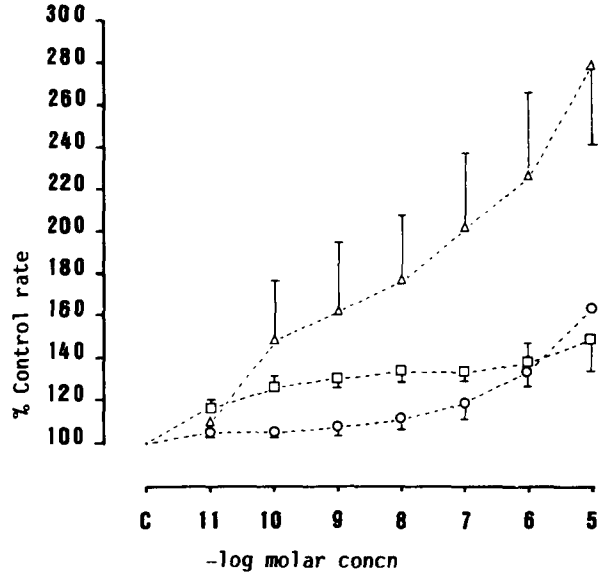

FIG. 2. Concentration-response curves for the effects of isoprenaline (△), noradrenaline (○) and phenylephrine (□) on ventricular automaticity. Each drug was applied cumulatively. The results are expressed as percentages of the control frequency recorded in the minute that preceded the addition of the drug (pre-drug period) into the bath. Each point represents the mean ± s.e. (vertical bars) of five experiments.

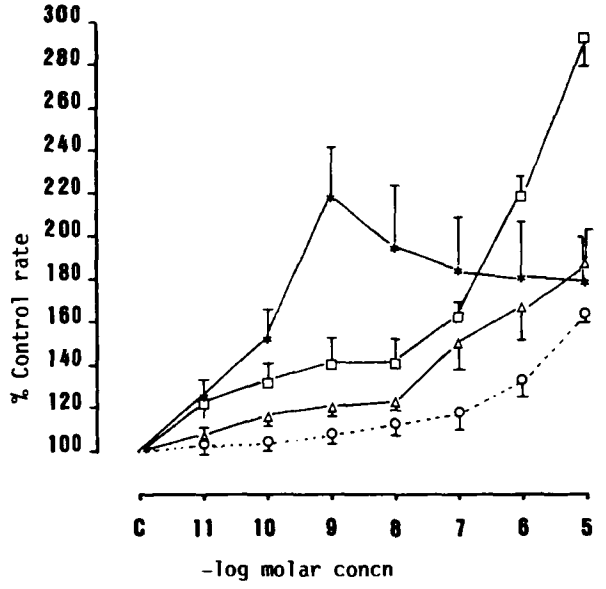

FIG. 3. Effects of noradrenaline on ventricular automaticity in the absence (○) and in the presence of 0·1% v/v of halothane (△), isoflurane (*) and enflurane (□). Each point represents the mean ± s.e. (vertical bars) of five experiments.

ventricular rate returned progressively to the control values. The increment of ectopic ventricular automaticity was dose-dependent but isoprenaline (n = 5) was consistently more effective than noradrenaline (n = 5) or phenylephrine (n = 5) in quantitative terms (Fig. 2). The maximal increase induced by isoprenaline (10^{-5} M) was 179 ± 39·6% ($P < 0·05$). The highest concentration tested (10^{-5} M) of phenylephrine and noradrenaline raised ventricular automaticity by 49 ± 16·9% and 64 ± 2·4%, respectively, when compared with the control

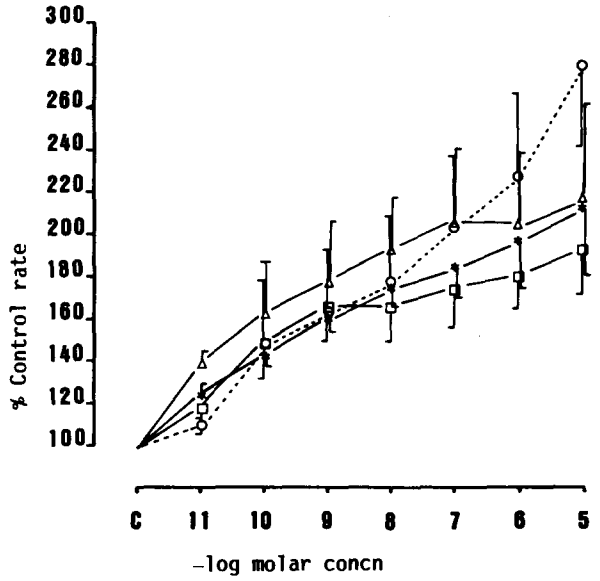

FIG. 4. Effects of isoprenaline on ventricular automaticity in the absence (○) and in the presence of 0·1% v/v of halothane (△), isoflurane (*) and enflurane (□). Each point represents the mean ± s.e. (vertical bars) of five experiments.

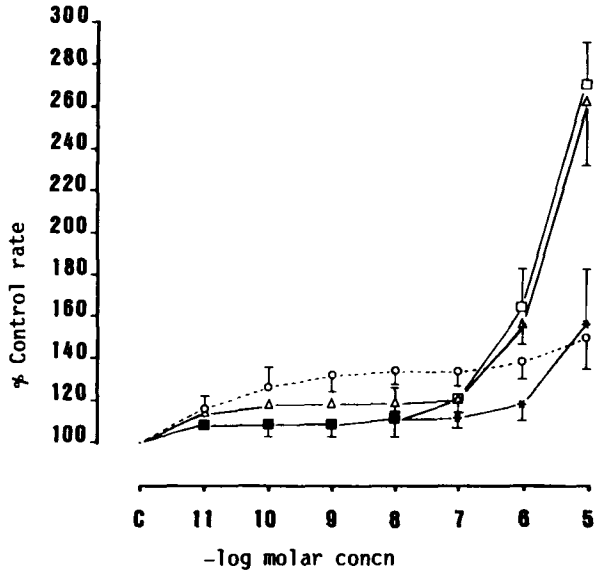

FIG. 5. Effects of phenylephrine on ventricular automaticity in the absence (○) and in the presence of 0·1 v/v% of halothane (△), isoflurane (★) and enflurane (□). Note the superimposition of points for enflurane and isoflurane (10^{-11} M–10^{-8} M). Each point represents the mean ± s.e. (vertical bars) of five experiments.

frequency, but the difference between the two values was not statistically significant.

However, the maximal effect of isoprenaline was significantly different from those of the other two amines ($P < 0.05$). Similar findings were obtained after statistical comparison of the ED50 values of the three drugs. The mean ED50 value in the isoprenaline group was $3.58 \pm 0.59 \times 10^{-10}$ M which was significantly lower ($P < 0.01$) than those of noradrenaline ($8.79 \pm 1.14 \times 10^{-7}$ M) and phenylephrine ($5.72 \pm 1.04 \times 10^{-7}$ M).

Inhalatory anaesthetics and noradrenaline

To know whether the anaesthetics would modify the effects of noradrenaline on ventricular automaticity, we made concentration–response curves to noradrenaline in the presence of each of the anaesthetics at 0·1% v/v. The results are summarized in Fig. 3. The concentration used was did not affect ventricular automaticity, but it did increase the effect of noradrenaline on cardiac automaticity. The maximal increment of ventricular automaticity induced by noradrenaline when compared with the control rate was 118 ± 19·0% and 193 ± 13·3% in the presence of isoflurane and enflurane, respectively. The differences between these two values and the maximal increase of ventricular automaticity induced by noradrenaline on its own were statistically significant ($P < 0.01$). Halothane also increased the maximal effect of noradrenaline (64 ± 2·4% in the absence, and 86 ± 17·3% in the presence, of halothane), but this increment was not statistically significant. The ED50 of noradrenaline ($8.79 \pm 1.14 \times 10^{-7}$ M) was significantly reduced ($P < 0.01$) in the presence of each anaesthetic being $4.8 \pm 2.6 \times 10^{-8}$ M, $4.4 \pm 0.7 \times 10^{-9}$ M, and $1.8 \pm 0.67 \times 10^{-8}$ M in the presence of halothane, isoflurane and enflurane, respectively.

Inhalatory anaesthetics and isoprenaline

Fig. 4 is a concentration–response curve for isoprenaline obtained in the absence and presence of 0·1% v/v of the anaesthetics. The maximal increment of ventricular rate induced by isoprenaline was 116 ± 45·6% (with halothane), 112 ± 33·7% (with isoflurane), and 92 ± 26·5% (with enflurane). These values were not statistically different when compared with the maximal effect of isoprenaline alone (179 ± 39·6%). The differences between the ED50 of isoprenaline in the absence and presence of each anaesthetic were also not significant.

Inhalatory anaesthetics and phenylephrine

Dose–response curves for phenylephrine were constructed in the presence of 0·1% v/v of the anaesthetics. The maximal effect with the highest concentration of phenylephrine (10^{-5} M) was 56 ± 28·3% after halothane, 161 ± 29·4% with isoflurane and 170 ± 20·6% with enflurane (Fig. 5). The maximal effect of phenylephrine alone (49 ± 16·9%) was significantly lower than the values obtained in the presence of isoflurane or enflurane ($P < 0.01$). Nevertheless, no statistical difference was found between the ED50 of phenylephrine in the absence and presence of any anaesthetic.

Discussion

It is generally accepted that catecholamines produce cardiac arrhythmias through the β-adrenoceptors in the heart and also because propranolol is particularly effective in suppressing these arrhythmias (Szekeres & Papp 1980). Nevertheless, there is recent evidence that α-receptors are also involved in the arrhythmogenic effect (Williams et al 1978). In our results, isoprenaline, which is devoid of α-adrenoceptor

activity was more effective than either noradrenaline or phenlyephrine in increasing ventricular automaticity. This is consistent with results showing that isoprenaline increases pacemaker or ectopic activity more effectively than adrenaline or noradrenaline (Dresel & Duncan 1961; Trautwein 1963). Thus, β-adrenoceptors seem to play a more significant role than α-adrenoceptors in this effect. The probable mechanism involved is the control of potassium conductance via β-adrenoceptors which increase the rate of the slow diastolic depolarization (phase 4) of the automatic cells (for review see Hauswirth & Singh 1979).

It is well known that the anaesthetics examined sensitize the heart to the dysrhythmic action of catecholamines (Katz & Epstein 1968; Munson & Tucker 1975). However, the type of adrenoceptor mediating this action is not known. The results presented here indicate that halothane, isoflurane or enflurane sensitized the myocardium to the effect of noradrenaline on ventricular automaticity by lowering the ED50 for noradrenaline. This result is at variance with in-vivo studies in which halothane sensitized the heart more extensively than isoflurane to the arrhythmogenic effect of exogenously administered catecholamines (Joas & Stevens 1971). This is probably related to the different effect induced in-vivo by the two anaesthetics on atrioventricular conduction. Halothane slows conduction through the A–V node and subsidiary pacemakers respond to increased adrenergic activity, or to exogenously administered catecholamines, by establishing either ventricular extrasystoles or tachycardia (Prys-Roberts 1987). Isoflurane, however, has the opposite effect on atrioventricular conduction (Marshall & Wollman 1985) and this could protect against arrhythmogenesis. In fact, pacing the heart at a sufficiently fast rate can override catecholamine-induced ventricular arryhthmia in the presence of anaesthetics (Hashimoto & Hashimoto 1972). Obviously, in our in-vitro preparation atrioventricular conduction does not play any role.

The inhalation anaesthetics tested did not modify the ED50 of isoprenaline or phenylephrine. That the specific β-agonist isoprenaline was not potentiated, in our results, implies that selective stimulation of β-adrenoceptors is not responsible for the enhancement of cardiac automaticity induced by catecholamines in the presence of the anaesthetics.

The existence of α-adrenoceptors mediating some cardiac properties such as positive inotropism and chronotropism has been demonstrated (Brückner et al 1985). It appears that α-adrenoceptor stimulation is also involved in the development of cardiac dysrhythmias. For example, phentolamine and prazosin significantly reduced both ventricular ectopic activity and mortality induced by coronary reperfusion following occlusion of the coronary artery in cats (Sheridan et al 1980). This finding has been confirmed in dogs in which prazosin also reduced mortality caused by ventricular fibrillation following reperfusion (Avory et al 1985). It has been suggested that α-adrenoceptors play a preferential role in the catecholamine-anaesthetic arrhythmias because a progressively less arrhythmogenic activity of adrenaline has been observed after a stepwise increase in α-adrenoceptor blockade (Maze et al 1985). Our data, however, are at variance with this view because the ED50 of phenylephrine (a powerful α-receptor stimulant) is not modified in the presence of the anaesthetics. From the interaction between noradrenaline and the anaesthetics in our results it could be inferred that a combined action of both α- and β-adrenoceptors are responsible for this effect. This is in agreement with previous experimental results showing that both α_1- and β_1-adrenoceptors are required for the full expression of the effect of halothane in sensitizing the heart to exogenously-administered catecholamines (Maze & Smith 1983; Hayashi et al 1986). This is also consistent with the fact that the anaesthetics potentiate the effect only at the highest concentration (10^{-5} M) of phenylephrine in our experiments. This α-agonist also has a weak β-agonist activity which becomes more evident at such concentrations (Weiner 1985).

It is difficult to determine whether α-adrenoceptors contribute to the arrhythmogenic effect of catecholamines by a direct mechanism of action on the myocardium. It has been suggested that haemodynamic effects on peripheral vasculature mediated by α-adrenoceptors are responsible for the arrhythmogenic interaction between adrenaline and volatile anaesthetics (Zink et al 1975). On the other hand, it has been claimed that a direct action on myocardial α-receptors explains such an effect (Maze et al 1985). Nevertheless, the major experimental problem here is the dissociation of α-adrenoceptor mediated effects on haemodynamics, the coronary vasculature and the myocardium (Dave 1986). Our experimental model precluded coronary and haemodynamic factors. Thus our results indicate a direct effect of α-agonists on the myocardium. The mechanism by which α-adrenoceptors raise cardiac automaticity has yet to be identified. The enhancement of calcium influx into myocardial cells has been suggested (Kimura et al 1984). Inhalation anaesthetics could also alter the uptake and release of calcium from the sarcoplasmic reticulum (Price & Ohnishi 1980).

In conclusion, isoprenaline was more potent than either noradrenaline or phenylephrine in increasing ventricular automaticity induced by a local injury of the isolated right ventricle of the rat. Thus, while β-adrenoceptors play a role in this effect, the anaesthetics sensitized the myocardium to the effect of noradrenaline, but not to that of isoprenaline, on ventricular automaticity. Therefore, simultaneous activation of both α- and β-adrenoreceptors seems to be indispensible to this sensitization.

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