Mechanisms of the Atrium-Specific Positive Inotropic Activities of Quinidine- and Atropine-Like Agents in Rats

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ABSTRACT

This study investigated the mechanism of the positive inotropic effects of class 1 antiarrhythmic agents using electrically stimulated right atria (sinoatrial node excised), left atria and right ventricles of rats. Quinidine, disopyramide and procainamide produced concentration-dependent positive inotropic effects on right and left atria; effects on the right atria were greater than on left atria. At concentration producing positive inotropic effects on atria, the contractions of right ventricles were slightly increased by quinidine, unaffected by disopyramide and decreased by procainamide. The positive inotropic effects of quinidine were inhibited by propranolol, reserpine and mecamylamine but not by cocaine, hexamethonium and 6-tubocurarine; propranolol also antagonized the positive inotropic effects of disopyramide and procainamide. Bupivacaine, which like quinidine blocks transient outward potassium current, slightly increased the contractions of right atria but not of left atria and ventricles. The atrium-specific positive inotropic effects of quinidine were mimicked by atropine, pirenzipine and dimethylphenylpiperazinium but not by nicotine, cytisine and butyrylcholine; the effects of atropine, dimethylphenylpiperazinium and pirenzipine were also blocked by propranolol. Quinidine increased the release of norepinephrine from atria but not from the ventricles; this release was greater from the right than from the left atria. It is concluded that quinidine- and atropine-like agents exert atrium-specific positive inotropic effects by blocking muscarinic receptors and permitting a dominance of acetylcholine effects via a release of norepinephrine from sympathetic nerve terminals.

Class 1 antiarrhythmic agents, quinidine, disopyramide and procainamide modify myocardial refractory period, conduction velocity and excitability by blocking sodium channels (Roden, 1996); in addition they all possess atropine-like activity to varying degrees (Corr et al., 1978; Mirro et al., 1980; Roden, 1996). Quinidine also inhibits various K+ currents (Imazumi and Giles, 1987; Snyder et al., 1992; Roden, 1996). Quinidine is the oldest antiarrhythmic agent. One of the problems in the clinical use of quinidine in atrial flutter or fibrillation is the potential of increase in ventricular rate because of a decrease in atrioventricular block presumably because of its atropine-like activity (Roden, 1996).

In the course of using quinidine as a potassium channel blocker, we observed that it produced positive inotropic effects on atrial but not on ventricular preparations. A myocardial stimulant effects of quinidine has been reported previously. For example, quinidine was found to reverse the effects of vagal stimulation on the sinus rhythm of cats (Dale, 1921) and dogs (Lewis et al., 1921) and it was concluded by Dale (1921) that the “partial or complete paralysis of vagal action produced by quinidine is not due to an atropine-like action.” A later study identified the dependence of the accelerator effects of quinidine on dog heart on norepinephrine (Roberts et al., 1962). However, the precise mechanism for the region-specific positive inotropic effects of quinidine is not known.

All regions of the heart are sympathetically innervated; however, cholinergic innervation is far more abundant in the atria than in the ventricles (Burnstock, 1969; Kent et al., 1974). We postulated that a positive inotropic effect of quinidine on atria but not on the ventricles might be related to its atropine-like action and relatively abundant cholinergic innervation of the atria but not of the ventricles. If so, endogenously released ACh might not be able to exert a negative inotropic effect via muscarinic receptors in the presence of quinidine but it might release endogenous norepinephrine from the sympathetic nerve endings via nicotinic ACh receptors. If this hypothesis was correct, a positive inotropic effect of quinidine will be exerted indirectly via norepinephrine and

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ABBREVIATIONS: ACh, acetylcholine; DA, dopamine; Epi, epinephrine; NE, norepinephrine; DMPP, dimethylphenylpiperazinium; nAChR, nicotinic acetylcholine receptors; RA, right atria; LA, left atria; RV, right ventricles; EDTA, ethylenediamine tetraacetate; pD2, negative log of the molar concentration of drugs producing 50% of the maximal effect.
other class 1 antiarrhythmic agents with significant atropine-like activity; as well, atropine and other antimuscarinic agents should exert positive inotropic effect on the atria but not on the ventricles. In our study we tested this hypothesis using electrically stimulated left and right atrial (devoid of sinus node) as well as right ventricular muscle preparations from rats.

Materials and Methods

Chemicals. Cocaine hydrochloride was purchased from BDH, Toronto, Ontario, Canada. The following agents were purchased from Sigma Chemical Co., St. Louis, MO: atropine sulfate, bufapacaine hydrochloride, cytosine, dimethyl-4-phenylpiperazinium iodide, dihydroxybenzylamine, disopyramide phosphate, L-dopamine, L-epinephrine, hexamethonium bromide, mecamylamine hydrochloride, nicotine sulphate, L-norepinephrine, procainamide hydrochloride, dihydroxybenzylamine, quinidine sulfate, reserpine, d-tubocurarine chloride, tyramine hydrochloride.

Animals. Male (250-350 g) Sprague-Dawley rats (Charles River, St. Constant, Quebec, Canada) were used according to a protocol of the McGill University Animal Care Committee. Animals were maintained at 23°C, 50 to 70% humidity and a 12-hr light-12-hr dark schedule (lights on 07.00-19.00 hr) and fed ad libitum rat food and tap water. To deplete endogenous norepinephrine, 5 mg/kg reserpine were injected i.p. 24 hr before animals were used for these studies. Rats were decapitated and hearts quickly removed and used for different experiments as described below.

Inotropic responses. Left atrial, right atrial and right ventricular strips were used to determine inotropic responses. Right atria were excised so as to exclude the sinoatrial node; if right atria exhibited spontaneous contractions, preparations were discarded. Right ventricles were cut along their length into three identical pieces, each approximately 3 x 10 mm; one or two of these strips were used. Atrial and ventricular preparations were set up in tissue baths at 32°C in Krebs buffer of the following composition (mM): NaCl 117, KCl 4.7, CaCl2 1.8, MgSO4 1.18, KH2PO4 1.2, NaHCO3 25, dextrose 11 and EDTA 0.03 (Varma and Yue, 1986; Deng et al., 1996). The buffer was gassed with a mixture of 95% O2 and 5% CO2. Preparation were stimulated at 1 Hz, 5 msec pulse duration and 1.5 times the threshold voltage (20-30 V). In each preparation, the tension was adjusted to yield maximal basal isometric contractions. The applied tensions to the atrial and ventricular preparations were approximately 0.5 and 1.0 g, respectively. The tension was recorded by means of Grass force-displacement transducers (FT03C) on a Grass polygraph (Quincy, MA). Preparations were allowed to equilibrate for 45 to 60 min with changes in Krebs buffer every 15 min. Inotropic effects of various agents were determined by cumulative increases in their concentrations.

To test the influence of other agents on positive inotropic responses, right atria were divided into two and set up as described above. One of these preparations served as the control and to the other was added propranolol, mecamylamine, hexamethonium, d-tubocurarine or cocaine 30 min before starting the construction of concentration-response curves to different agents; these agents were left in the bath during the construction of concentration-response curves to inotropic agents since their effects can be reduced by washout. Inotropic responses were calculated as changes in the contractile force, which existed just before starting the construction of concentration-response curves to test agents.

The experimental condition described above was selected for determining inotropic responses because the contractions of myocardial preparations were smaller and less stable at the body temperature of 37°C than at 32°C. Also basal contractions decreased as the stimulus frequency was increased, e.g., the force of basal contractions at 4 Hz (far less than the usual heart rate of 400 beats per min of conscious rats) was approximately 20% of that at 1 Hz. EDTA was added because it is expected to reduce spontaneous oxidation of catecholamines by chelating residual trace metals that might be present in distilled-deionized water. Although quinidine at maximally effective concentrations did increase the contractions of the left atria (basal 200 ± 29 mg) to 142 ± 14% of the basal and of the right atria (basal contractions 181 ± 48 mg) to 172 ± 23% of the basal at 37°C, 3 Hz and zero EDTA (n = 4), a temperature of 37°C and a frequency higher than 1 Hz was considered unsuitable for these studies; all studies described here were therefore done at 32°C and 1 Hz in the presence of EDTA.

Effect of quinidine on catecholamine release. Right and left atrial strips were set up as described above but in 20 ml buffer. Preparations were equilibrated for 45 min and the buffer was changed; 1 μM cocaine was present throughout to inhibit uptake of released norepinephrine. A 15-min sample of the buffer (20 ml) was collected in 50 ml polypropylene tubes and the pH of the buffer immediately reduced to 5 by adding predetermined volume of 1 M HCl. The buffer was changed and 1 μM quinidine was added and a 15-min sample of the buffer was collected and acidified as before; this process was repeated with increasing concentrations (3, 30, 300 μM) of quinidine; after the last buffer collection, the wet weights of tissues were recorded. The release of catecholamines from right ventricular strips was determined following a single 30 μM concentration of quinidine. Buffer samples were stored at -20°C for the assay of catecholamines within less than 1 wk.

Epinephrine, norepinephrine and dopamine were assayed by high-pressure liquid chromatography (Waters Pump model 510) using electrochemical detectors (Waters model 460, Milford, MA) as described (Shohami et al., 1983). Dihydroxybenzylamine was used as the internal standard and added into the assay samples and then adsorbed to activated alumina. Catecholamines were eluted from the alumina with 150 μl of 0.1 M phosphoric acid; an aliquot of this was injected via a WISP (M712, Waters) injector into a BioRad (BioRad, Richmond, CA) silica-based weak cation exchange column (195-6003) with a guard column (195-6003) using a 0.07 M citric acid-acetoni- trile (88:12) solvent at a flow rate of 1.3 ml/min. Catecholamines were quantified on the basis of the ratio of the peak height generated by authentic amines to that by the internal standard. Under the condition of these assays, the retention times for epinephrine, norepinephrine, dopamine and the internal standard dihydroxybenzylamine were 2.85, 3.8, 4.25 and 4.85 min, respectively. The electrochemical detector was set at 0.58 V. The detection limit of epinephrine was 20 fmol and of norepinephrine and dopamine 60 fmol. Intraassay and interassay variations were 3.7 ± 1.7% and 7.5 ± 5.0%, respectively. In four additional experiments 10 nM norepi- nephrine was added to the bath in the absence of any tissue; the recovery of norepinephrine under these conditions was 92 ± 1.7%.

Statistics. Data were compared by Student’s t test for unpaired or paired data and a P < .05 was assumed to denote significant differences. Data are presented as mean ± S.E.M.

Results

Basal contractions and effects of pretreatments. Basal contractions of all the right atria, left atria and right ventricles used in this study were 330 ± 15 mg (n = 98), 421 ± 17 mg (n = 100) and 651 ± 38 mg (n = 53), respectively, and significantly (P < .05) different from each other. However, there were marked variations in basal contractions so that contractions of left and right atria used to test different agents did not always differ significantly (table 1). The basal contractions of right and left atria from reserpine treated rats were 290 ± 23 mg (n = 14) and 361 ± 31 mg (n = 12), respectively, and did not differ significantly from the corresponding basal contractions of these tissues (right atria 330 ± 15 mg, n = 98; left atria 421 ± 17 mg, n = 100) from
and procainamide (fig. 1c) produced concentration-dependent
increases in the contractile force of right and left atria (table 1); effects became apparent within 1 to 2 min and reached a peak in 5 to 10 min. The concentration-response-curves were relatively steep and the difference between the minimal and maximal concentrations were usually 10-fold. Contractions returned to basal levels after a 60- to 90-min period of repeated wash; a second concentration-response curve to quinidine did not reveal any apparent “tachyphylaxis.” The contractions of right ventricles were slightly but significantly (P < .05) increased by quinidine, not modified by disopyramide and significantly (P < .05) decreased by procainamide (table 1). Bupivacaine exerted a slight but significant (P < .05) decrease in the basal contractile force of rat right atrial strips at ASPET Journals on October 15, 2017 jpet.aspetjournals.org Downloaded from
.05) positive inotropic effect on the right atria but significantly (P < .05) inhibited the contractions of the left atria and the right ventricles (fig. 1d; table 1).

At 100 μM and higher concentrations, quinidine produced a negative inotropic effect on the atria and ventricles. The depressant activity of quinidine was followed in five experiments. The contractions of right atria decreased to 75 ± 10% of the maximal in 10 ± 1 min after 300 μM quinidine, to 52 ± 9% in 7 ± 0.6 min after 500 μM quinidine and to 0% in 12 ± 1 min after 700 μM quinidine; the time-course and dose-dependence of the depressant effects of quinidine on the left atria were very similar to that on the right atria. However, quinidine was more effective in depressing right ventricular preparations; contractions decreased to 82 ± 6% of maximal in 11 ± 0.5 min after 100 μM, to 20 ± 9% in 10 ± 1.1 min after 300 μM and to 0% in 8 min after 500 μM quinidine.

**Inotropic response to isoproterenol.** As with the class 1 antiarrhythmic agents, isoproterenol also produced greater maximal positive inotropic effect on the right atria than on the left atria; as expected isoproterenol increased the force of contractions of right ventricular strips (table 1). Indeed, of all the agents studied (isoproterenol, quinidine, disopyramide, procainamide, bupivacaine, atropine, pirenzepine, nicotine, DMPP, cytisine, butyrylcholine and physostigmine), only isoproterenol and to a much smaller extent quinidine exerted positive inotropic effect on right ventricular strips (table 1).

**Fig. 2.** Effects of reserpine and of propranolol on the positive inotropic responses of electrically (1 Hz) driven right atrial strips from rats to quinidine (a and c) and disopyramide (b and d). Reserpine (5 mg/kg, i.p.) was injected 24 hr before experiments. To determine the effect of propranolol, right atria were divided into two; one served as the control and the other was treated with 1 μM propranolol (30 min contact). Data are mean ± S.E.M. of 6 to 12 experiments.

**Fig. 3.** Effects of atropine (a), pirenzepine (b), DMPP (c) and nicotine (d) on the force of contractions of rat right atrial (○), left atrial (■) and right ventricular (△) strips stimulated at 1 Hz. Sinus node was excised such that none of right atria exhibited spontaneous contractions. Data are means ± S.E.M. of six to eight experiments.
Effects of reserpine and propranolol on responses to quinidine and disopyramide. As stated above, pretreatment with reserpine did not produce significant effects on the basal contractions of atrial preparations. However, reserpine pretreatment significantly decreased the positive inotropic effects of both quinidine (fig. 2a) and disopyramide (fig. 2b).

Propranolol (1 μM) significantly decreased the contractions of right atria to 76 ± 4% of the basal. Propranolol antagonized the positive inotropic effects of both quinidine (fig. 2c) and disopyramide (fig. 2d) and significantly (P < .01) decreased the maximal effects of both these agents. For example, the maximal contractile force of right atria after 30 μM quinidine was 270 ± 31% of the basal in the absence and 131 ± 10% of the basal in the presence of 1 μM propranolol (n = 10); similarly, the maximal contractile force of right atria after 30 μM disopyramide was 214 ± 24% of the basal in the absence and 154 ± 9% of the basal in the presence of propranolol (n = 5). The effect of propranolol on the concentration-response curves to procaïnamide was not studied; however, the maximal positive inotropic response to procainamide was decreased from 203 ± 4% of the basal to 80 ± 5% of the basal following the addition of 1 μM propranolol (n = 5).

Effects of cholinergic and anticholinergic agents on myocardial contractions. Atropine (fig. 3a), pirenzepine (fig. 3b) and DMPP (fig. 3c) produced a concentration-dependent increase in contractions of the left and right atria but not of the right ventricles; the positive inotropic effects on the right atria were greater than on the left atria. Nicotine (fig. 3d), cytisine and butyrylcholine did not produce positive inotropic effects on any of the myocardial preparations (table 1). Physostigmine and butyrylcholine decreased the contractions of atria but exerted little effect on the contractions of the right ventricles (table 1).

Antagonism of the positive inotropic effects of atropine, DMPP and pirenzepine by propranolol. Propranolol (1 μM) antagonized the positive inotropic effects of atropine (fig. 4a) and DMPP (fig. 4b) and significantly (P < .01) decreased the maximal effects of both these agents. For example, the maximal contractile force of right atria after 1 μM atropine was 197 ± 26% of the basal in the absence and 125 ± 13% of the basal in the presence of 1 μM propranolol (n = 6); similarly, the maximal contractile force of right atria after 30 μM DMPP was 194 ± 13% of the basal in the absence and 154 ± 8% of the basal in the presence of propranolol (n = 5). The effect of propranolol on the concentration-response curve to pirenzepine was not studied; however, the peak response to pirenzepine was significantly (P < .01) reduced from 218 ± 23% of the basal to 101 ± 5% of the basal after the addition of 1 μM propranolol (n = 5).

Effects of cocaine and nicotinic ACh receptors antagonists on inotropic responses to quinidine. Cocaine (1 μM) cause a slight but insignificant increase in basal contractions and did not inhibit the positive inotropic effects of quinidine (fig. 5a); at this concentration cocaine markedly reduced the positive inotropic effects of tyramine (data not shown).

Mecamylamine, hexamethonium and d-tubocurarine did not exert any obvious effects on basal contractions. The positive inotropic response to quinidine was inhibited by mecamylamine; 10 μM mecamylamine shifted the positive inotropic dose ratio by 1.8 ± 0.01 (n = 4) and 100 μM mecamylamine suppressed the maximal effect of quinidine from a control value of 202 ± 31% of the basal to 140 ± 12% of the basal (fig. 5b). Up to concentrations of 100 μM, hexamethonium (fig. 5c) and d-tubocurarine (fig. 5d) did not inhibit the inotropic effects of quinidine.

Effects of quinidine on catecholamine release. Both atria and ventricles released norepinephrine, epinephrine as well as dopamine in the absence of quinidine (basal release); the release of norepinephrine was greater than that of the other two catecholamines (fig. 6). Quinidine caused a concentration-dependent increase in norepinephrine release from both the right (fig. 6a) and left (fig. 6b) atria; however, the release of norepinephrine declined to basal levels following the highest concentration (300 μM) of quinidine tested. Quinidine did not significantly increase the release of epinephrine and dopamine. At 30 μM concentration, which caused maximal release of norepinephrine from both the left and right atria, quinidine did not cause a significant release of any of the catecholamines from right ventricular strips (fig. 6c).

Discussion

Diverse agents can cause myocardial depression and this is often described as “quinidine-like” effect. Therefore a positive inotropic activity of quinidine as found in our study might seem unusual. However, a stimulant effect of quinidine on cardiac rate (Dale, 1921; Lewis et al., 1921; Roberts et al., 1962) and a positive inotropic effect of quinidine on atria but not on ventricular muscles of guinea pigs (Nawrath, 1981) has been previously reported. Our study confirms these observations and attempts to identify the underlying mechanisms. Specifically we tested the hypothesis that the atrium-specific positive inotropic effects of quinidine are related to its known atropine-like activity (Mirro et al., 1980; Roden,
and to the presence of abundant cholinergic plus noradrenergic innervation of atria (Burnstock, 1969) but sparse cholinergic innervation of the ventricles (Kent et al., 1974). A negative inotropic effect of physostigmine on the atria but not on the ventricles as found in our study (table 1) are consistent with the data that ventricles are poorly innervated by cholinergic nerves.

This study demonstrates that quinidine and the other class 1 antiarrhythmic agents, disopyramide and procainamide, significantly increase the force of contraction of atria at concentrations within the therapeutic range; the contractions of ventricles were slightly increased by quinidine, virtually unaffected by disopyramide and consistently decreased by procainamide (fig. 1; table 1). However, high concentrations of quinidine (100 μM) and procainamide (10 mM) decreased the contractile force of atria as well; indeed quinidine slightly
decreased the contractions of atria even at lower concentrations (1-3 μM) after the blockade of beta adrenoceptors with propranolol. It would thus appear that the positive inotropic effects determined in this study reflect the arithmetic sum of a stimulant and depressant activity with the former being dominant at low and the latter at high concentrations of the drug.

The quantitative differences between the positive inotropic efficacies of quinidine, disopyramide and procainamide most probably relate to their relative ACh muscarinic receptor blocking potencies with quinidine and disopyramide being more potent than procainamide (Mirro et al., 1980; Roden, 1986). The importance of antimuscarinic activity in the positive inotropic effects of class 1 antiarrhythmic agents is strongly supported by the observation that atropine and pirenzepine mimicked the effects of these agents (fig. 3). Pirenzepine is a selective M1 ACh receptor antagonist at low and M2 receptor antagonist at high concentrations and the heart contains low affinity muscarinic receptors of the M2 type (Hammer et al., 1980; Goyal, 1989). The positive inotropic effects of both atropine and pirenzepine were observed at a relatively high concentration range (0.1-10 μM) and pirenzepine was approximately 10-fold less potent than atropine (table 1); this is compatible with their relative M1 ACh receptor blocking potencies in atria (van Charldorp and van Zwieten, 1989).

Our data suggest that the positive inotropic effects of quinidine are produced indirectly by a release of norepinephrine; this inference is supported by two sets of observations. First, quinidine caused a concentration-dependent release of norepinephrine from both the right and left atria but not the ventricles and the maximal release coincided with its maximal positive inotropic effects (fig. 6). However, the positive inotropic effects of quinidine cannot be quantitatively explained on the basis of the net release of norepinephrine as measured in this study; it is reasonable to assume that a significantly higher concentration of neuronally released norepinephrine is delivered to the receptors than can be quantified by the technique used in this study. Second, the positive inotropic effects of quinidine were antagonized by propranolol and inhibited by pretreatment of rats with reserpine (fig. 2). Our inference is in conformity with an earlier study, which also suggested the role of sympathetic nervous system in the cardiac stimulant effect of quinidine in dogs (Roberts et al., 1962). Because quinidine, disopyramide and procainamide as well as atropine and pirenzepine produced little or no positive inotropic effect on ventricles (figs. 1 and 2), it is unlikely that their effects were mediated by a direct activation of adrenoceptors; indeed both quinidine (Roden, 1996) and atropine (Varma and Yue, 1986) possess antiadrenergic properties. A greater positive inotropic effect of these agents on the right than on the left atria seems to be due to a greater release (fig. 6a, b) as well as effect of norepinephrine in the former than in the latter tissue. Because isoproterenol also caused a greater increase in the force of contractions of the right than of the left atria (table 1), it would seem that the differences in the responses of the right and left atria are also related to the relative roles of beta adrenoceptors in the two organs.

Quinidine blocks several potassium currents (Roden, 1996) including the transient outward K+ current (Ito) (Imaizumi and Giles, 1987; Snyders et al., 1992) and prolongs action potential duration (Nawrath, 1981). It is thus possible that the positive inotropic activity of quinidine was partly caused by potassium channel blockade; a slight increase in the force of contractions of ventricles by quinidine and a lesser attenuation by reserpine of the effects of quinidine than of disopyramide support this possibility. It is possible that disopyramide, procainamide and atropine also block potassium channels although we are not aware of any such reports; if so the positive inotropic effects of these agents could also have been contributed by potassium channel blockade. As well, atropine could prolong atrial refractory period by its anti-muscarinic action. Notwithstanding these possibilities, overall data of this study suggest that potassium channel blockade is not central to the positive inotropic activities of quinidine- and atropine-like agents. Such marked disparity between the positive inotropic efficacy of quinidine on the atria and ventricles and significant inhibition of its effects by propranolol and reserpine suggest that the major mechanism of its positive inotropic activity is the release of norepinephrine. The data with bupivacaine lend support to the inference that the major effects of quinidine are exerted through a release of norepinephrine. Bupivacaine is very similar to quinidine as a blocker of transient outward K+ current (Courtney and Kendig, 1988; Castle, 1990); however, its positive inotropic effect on the right atria were significantly less than that of quinidine and it did not increase the force of contractions of the left atrial preparations.

This study provides strong evidence that the positive inotropic effects of quinidine are produced indirectly via a release of norepinephrine. However, the mechanisms of the release of norepinephrine by quinidine is not quite clear from our data. It is unlikely that quinidine directly releases norepinephrine from sympathetic nerve endings like tyramine (Potter and Axelrod, 1963); if it did so, one would expect quinidine to also exert a positive inotropic effects on the ventricles such as tyramine but this was not the case. Also, cocaine that inhibits the effects of indirectly acting sympathomimetic amines (Trendelenburg, 1966), did not inhibit the positive inotropic effects of quinidine. Indeed, quinidine-induced release of norepinephrine was measured in the presence of cocaine. Our data suggest that the release of norepinephrine by quinidine is mediated by ACh. Whether or not quinidine increases the release of ACh is not clear from our studies but atropine can release ACh (MacIntosh and Oborin, 1953; Goyal, 1989) and other atropine-like drugs such as class 1 antiarrhythmic agents might also do so. It can thus be surmised that the released ACh (enhanced or basal) acts on adrenergic nerve terminals to release norepinephrine. This suggestion is supported by the observation that quinidine increased the release of norepinephrine from atria but not ventricles (fig. 6). The inability of quinidine to release norepinephrine from the ventricles can be explained by an absence of abundant cholinergic innervation of the ventricles (Burnstock, 1969). This inference is also consistent with the results of functional studies that quinidine, atropine and other agents caused little or no increase in the contractile force of ventricles but produced a significant positive inotropic effect on the atria.

Assuming that ACh causes the release of norepinephrine from atrial adrenergic nerves, our data provide indirect evidence that this involves an unusual subtype of nAChR. For
example, atrial contractions were not increased by nicotinic agents nicotine, cytisine and butyrycholine. The inotropic effects of quinidine were not antagonized by hexamethonium and d-tubocurarine but inhibited by mecamylamine; mecamylamine is known to differ in certain respects from other nAChR antagonists (Bertrand et al., 1990). In other words, the indirect action of quinidine could neither be mimicked by classical nAChR agonists nor blocked by conventional antagonists. However, DMPP very closely mimicked the effects of quinidine; it exerted positive inotropic effects on the atria but not on the ventricles and its effects on the atria were blocked by propranolol suggesting that the increase in atrial contractions was caused by norepinephrine. These data are very similar to an earlier study that found that DMPP exerted a stimulant effect on rat atria, which was blocked by bretylium but not by hexamethonium (Chiang and Leaders, 1965).

Taken together data of this study suggest that the positive inotropic effects of various quinidine- and atropine-like agents are caused by a single mechanism that involves a release of norepinephrine from adrenergic nerve terminals by ACh acting on nAChR; these nAChR are responsive to DMPP but not to nicotine and cytisine. Indeed nAChR with α3β2 combination have been found to be equally sensitive to ACh and DMPP, much less sensitive to nicotine and virtually insensitive to cytisine (Luetje and Patrick, 1991); our data provide pharmacological evidence for the presence of such nicotinic receptors in the atria of rats. Most probably our assay system (inotropic response) is not sensitive enough to demonstrate a weak positive inotropic response to nicotine.

In conclusion our study demonstrates that cholinergic innervation of the atria and blockade of ACh muscarinic receptors are critical for the positive inotropic activity of quinidine. The blockade of muscarinic receptors is necessary to prevent the negative inotropic effect of ACh and cholinergic innervation is necessary for the ACh-mediated release of norepinephrine. In short, the atria-specific positive inotropic effects of class 1 antiarrhythmic and atropine-like agents are caused by ACh-mediated norepinephrine release from adrenergic nerve terminals via nAChR responsive to DMPP but not to nicotine and cytisine (possibly nAChR with α3β2 combination). If the data derived from rats reflect events in humans, the propensity of quinidine to decrease atrioventricular block and increase ventricular rate during atrial flutter or fibrillation, might not be entirely due to its direct antimuscarinic activity but also contributed by a release of norepinephrine. Also, the high risk of ventricular fibrillation after the clinical use of atropine to treat bradycardia (Massumi et al., 1972; Richman, 1974) or in animal experiments (Corr and Gillis, 1974) might in part be contributed by a release of norepinephrine.

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References


