Interaction between Alpha-1 Adrenergic and Vagal Effects on Cardiac Rate and Repolarization

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ABSTRACT

Alpha-1 adrenergic stimulation modulates ventricular automaticity via an alpha-1 adrenoceptor (AR) subtype blocked by the alpha-1B antagonist chloroethylclonidine (CEC) and alters repolarization via receptor subtype(s) (alpha-1A and alpha-1D) blocked by WB4101. Our objective was to determine alpha-1 AR subtype specific effects and vagal interactions on heart rate and ventricular repolarization. We studied right vagally innervated Langendorff-perfused guinea pig hearts, beta-blocked with propranolol, 5 x 10⁻⁷ M. Heart rate and QT interval were measured from bipolar epicardial electrodes. In some experiments rate corrected QT interval (QTc) (Bazett formula) was calculated, as well. Phenylephrine (PE) alone, 10⁻⁷ M, reduced sinus rate significantly (P < .05) in 8 of 13 preparations. A decrement in rate occurred in all preparations in the presence of WB4101 and was blocked by CEC. Vagal stimulation, at 1 to 20 Hz slowed heart rate (P < .05) in a frequency-dependent fashion. Addition of PE alone or in the presence of WB4101 further reduced rate (P < .05). However, with vagal stimulation + PE + CEC, rate did not differ from that in the presence of vagal stimulation, alone (P > .05). In studies of repolarization, QTc shortening was elicited by PE alone (P < .05) and CEC + PE (P < .05). In the presence of WB4101, no QTc shortening occurred (P > .05). QTc shortening induced by vagal stimulation was attributable to the heart rate change rather than to a direct effect on ventricular repolarization. In conclusion, in the setting of beta adrenergic blockade, an alpha-1B receptor appears responsible for the alpha-1 adrenergic decrease in heart rate and facilitation of vagal responsiveness. A receptor subtype blocked by WB4101 (alpha-1A or alpha-1D) is responsible for the QT and QTc shortening. Whereas right vagal stimulation shortens the QTc interval, this action reflects the change in sinus rate rather than an effect on the ventricle.

The alpha-1 receptor subtype blockers WB4101 (alpha-1A and alpha-1D) and CEC (alpha-1B) are frequently used to distinguish physiological effects of alpha-1 receptor stimulation (for review, see Minneman, 1988; Terzic et al., 1992). In normal cardiac Purkinje fibers stimulation of a WB4101-sensitive alpha-1 receptor subtype increases ventricular automaticity and prolongs repolarization (Lee et al., 1991). In contrast, stimulation of a chloroethylbenzamine-sensitive alpha-1 subtype activates the Na/K pump via signal transduction dependent on a PTX-sensitive G protein (Shah et al., 1988). This pathway decreases intracellular Na activity, slows automatic rate, and accelerates repolarization (Zaza et al., 1990). The balance between these two subtype actions determines the net electrophysiologic response to alpha-1 adrenergic stimulation.

Although little has been done to explore alpha-1 receptor-vagal interactions, some potential importance of these is implied by the vagal and beta adrenergic interactions on the heart. Vagal stimulation has negative chronotropic, dromotropic and inotropic effects, respectively, at sinoatrial, AV nodal and myocardial sites (Loeffelholz and Pappano, 1985). The effects of acetylcholine at atrial and AV nodal levels can occur in the presence or absence of sympathetic innervation and/or beta adrenergic catecholamines (Carrier and Bishop, 1972). Particularly important to the effects of acetylcholine on supraventricular tissues and, perhaps, essential to its actions on ventricle is its antagonism of the receptor-effector coupling pathway of beta adrenergic catecholamines (Levy et al., 1972). This “accentuated antagonism” of beta adrenergic actions is based on acetylcholine’s effect to reduce adenylate cyclase activation via a G₂-transduced pathway. This reduces the action of beta adrenergic receptor stimulation to initiate effector responses transduced via the GTP regulatory protein Gₛ and adenylate cyclase activation (Robishaw and Foster, 1989).

That there is a basis for expecting vagal alpha-1 adrenergic interactions is suggested by the work of Wendt and Martins (1990) in intact canine hearts beta blocked with metoprolol. Phenylephrine increased the Purkinje fiber relative refractory period and vagal stimulation enhanced the increase. No such changes were observed in ventricular endocardial mus-

ABBREVIATIONS: QTc, rate corrected QT interval; CEC, chloroethylbenzamine; WB, WB4101.
cule refractory periods. Vagal alpha-1 adrenergic interactions also have been demonstrated in isolated rat atria, in which there is alpha-1 adrenergic inhibition of vagal ACh release (Wetzel et al., 1985). Such inhibition of the vagus would facilitate increases in heart rate during sympathetic stimulation.

Based on this background, our study was designed to assess the effects of alpha-1 receptor subtype stimulation on sinoatrial rate and ventricular repolarization of vagally innervated, isolated guinea pig hearts. Using this model, we studied 1) the effects of phenylephrine perfusion and of vagal stimulation individually on sinoatrial rate and ventricular repolarization and 2) the modulation of alpha-1 adrenergic effects WB4101 and CEC.

Methods

Male guinea pigs (weight 250–300 g) were anesthetized with i.p. sodium pentobarbital (30 mg/kg). The heart was rapidly excised and via careful dissection the right vagus nerve was maintained intact from the thoracic inlet to the heart. We previously used this technique effectively to stimulate the vagi of Langendorff-perfused hearts in small animals (Shvilkin et al., 1994). The caveat concerning this procedure is that results are referable to the effects of right vagal stimulation only.

The ascending aorta was cannulated and each heart was retrogradely perfused with Krebs-Henseleit solution containing (in mM): NaCl, 100; KCl, 2.8; NaEDTA, 0.5; KH2PO4, 1.2; CaCl2, 3.0; glucose, 11.0; HEPES acid, 25; HEPES Na salt, 25, achieving a pH 7.4. Temperature was kept at 37°C by a glass heat exchanger and the hearts in small animals (Malfatto et al., 1985). We have previously used the formula in studies of linearity, it is considered to reasonably approximate the QT interval formula, especially at rapid heart rates where there is a loss of control, it was 8.9 ± 0.3 ml/min; for CEC 9.0 ± 0.4 ml/min and for WB4101, 9.7 ± 0.5 ml/min. The values for the three groups, respectively, in the presence of phenylephrine, 1 × 10−6 M, were 8.8 ± 0.3, 8.5 ± 0.1 and 9.6 ± 0.5 ml/min (all P > .05, n = 8 in all groups).

Effects of phenylephrine during vagal stimulation. Vagal stimulation at 1 to 20 Hz reduced sinus rate in a frequency-dependent fashion (fig. 2A). The reduction in pacemaker responsiveness to vagal stimulation was enhanced by

Results

Effects of phenylephrine on sinus rate. As we have demonstrated in earlier studies of ventricular specialized fibers (Rosen et al., 1977; Danilo, 1985), there were two populations of sinus node response to alpha agonist. Of 13 preparations studied, 8 showed a phenylephrine-induced decrease in sinus rate, and 5 showed no change (fig. 1). When phenylephrine was superfused in the presence of WB4101 or CEC, the uniform response of sinus rate was seen: all eight preparations manifested decreased automaticity. In contrast, the phenylephrine-induced decrease in sinus rate was attenuated by chloroethylclonidine of eight preparations, six showed no change in automaticity and two still showed a decrease (P < .05 cf WB: Fisher’s exact test). Finally, in the presence of both blockers, a response not unlike control was seen, although the magnitude of the decrease in sinus rate was reduced (data not shown).

Coronary flow did not vary significantly among groups: for control, it was 8.9 ± 0.3 ml/min; for CEC 9.0 ± 0.4 ml/min and for WB4101, 9.7 ± 0.5 ml/min. The values for the three groups, respectively, in the presence of phenylephrine, 1 × 10−6 M, were 8.8 ± 0.3, 8.5 ± 0.1 and 9.6 ± 0.5 ml/min (all P > .05, n = 8 in all groups).

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Effects of phenylephrine during vagal stimulation. Vagal stimulation at 1 to 20 Hz reduced sinus rate in a frequency-dependent fashion (fig. 2A). The reduction in pacemaker responsiveness to vagal stimulation was enhanced by
phenylephrine, with this enhancement best seen at 5 Hz (fig. 2A). To further explore the interaction between α agonist and vagal effect on sinus rate another set of experiments was performed in which vagal stimulation at 20 Hz was delivered and phenylephrine 10⁻²⁸ to 10⁻⁶ M was then perfused (fig. 2B). Seen more clearly here than with the protocol in figure 2A is the effect of phenylephrine to concentration-dependently reduce sinus rate over the effect seen with vagal stimulation alone. The response persisted in the presence of WB4101 and was attenuated by CEC.

**Effects of phenylephrine on ventricular repolarization.** In these experiments, two protocols were used. In the first, hearts were in sinus rhythm; in the second, they were paced from the right ventricle to maintain a constant rate 10% faster than their sinus rate. Results of the first protocol are presented in figure 3. As demonstrated in A, in the absence of vagal stimulation, phenylephrine shortened the QTc interval concentration dependently. The response persisted in the presence of chloroethylclonidine and was blocked by WB4101. As shown in B, vagal stimulation (20 Hz) significantly decreased QTc. This effect was also seen in the presence of CEC and was blocked by WB4101. *P < .05 cf control. *P < .05 cf C + VS.

![Fig. 2. A, Effects of vagal stimulation at 1 to 20 Hz alone and in the presence of phenylephrine, 10⁻²⁸ to 10⁻⁶ M on sinus rate. At ≥5 Hz all preparations showed decreased automaticity (*P < .05 cf control). At 5 Hz, only the decrease in the presence of phenylephrine > that with vagal stimulation, alone (P < .05). B, Effects of phenylephrine, alone (n = 6) and in the presence of CEC (n = 6) or WB4101 (n = 7) both 10⁻⁷ M, on sinus node responsiveness to vagal stimulation at 20 Hz. Neither CEC nor WB4101 alone significantly altered sinus rate. Vagal stimulation (VS) decreased sinus rate from a control value of approximately 205 bpm (C) to the values indicated as (C + VS). Phenylephrine further reduced the sinus rate beyond that seen with vagal stimulation alone. This decrease was comparable in the presence of WB4101, and was attenuated by CEC. *P < .05 cf control. *P < .05 cf C + VS.](image)

![Fig. 3. A, Effects of phenylephrine, alone (n = 5) and in the presence of CEC (n = 6) or WB4101 (n = 6), both 10⁻⁷ M on the QTc interval. Phenylephrine alone significantly reduced QTc. This effect was also seen in the presence of CEC and was blocked by WB4101. *P < .05 cf control. B, Effects of phenylephrine, alone (n = 6) and in the presence of CEC (n = 6) or WB4101 (n = 7), both 10⁻⁷ M on QTc during vagal stimulation. Vagal stimulation (C + VS) shortened QTc in all three groups (P < .05). Addition of phenylephrine, alone, or in the presence of CEC or WB4101 had no further effect.](image)

In the second protocol, the hearts were paced at a constant rate, 10% faster than their sinus rate. The pacing rates in the phenylephrine group, WB + phenylephrine group and CEC + phenylephrine group were, respectively 260 ± 10 (n = 5), 288 ± 14 (n = 4), and 290 ± 15 (n = 5) bpm (P > .05). Here, vagal stimulation at 20 Hz had no effect on the Q–T interval (control 182 ± 4 msec; vagal stimulation 183 ± 4 msec (n = 14) (P > .05).

Figure 4 shows the changes in QT interval during ventricular pacing. With vagal stimulation, phenylephrine shortened the QT interval. Significant reduction of the phenylephrine effect was achieved with WB4101 but not chloroethylclonidine. A comparable result was attained with
phenylephrine in the absence of vagal stimulation (data not shown). Because this result might indicate that either there was no significant right vagal innervation of the ventricle or that there was an inoperative $M_2$ muscarinic receptor-effector coupling system in the ventricle, we perfused eight additional, ventriculally paced hearts with acetylcholine, $10^{-7}$ to $10^{-5}$ M. Control QT $= 156 \pm 2$ msec. There was a slight and nonsignificant ($P > .05$) effect of acetylcholine on repolarization such that at $10^{-5}$ M, QT $= 146 \pm 2$ msec.

**Discussion**

There are conflicting data on alpha-1 adrenergic modulation of sinus rate. A negative chronotropic effect induced by methoxamine and phenylephrine has been reported in rabbit sinoatrial node cells (Satoh and Hashimoto, 1988), whereas data from isolated rat right atria indicate that phenylephrine induces a positive chronotropic effect antagonized by WB4101, but not CEC. There are two important aspects to the interactions of alpha-1 adrenergic and parasympathetic effects are complex. Studies of isolated rat hearts have shown that parasympathetic actions can be regulated through an alpha-1 adrenergic receptor having different effects at pre- and postganglionic levels (Pardini et al., 1991). An alpha mediated facilitation of acetylcholine release in the perfused rat heart has also been reported (Bognar et al., 1990) and, in contrast, alpha-1 mediated inhibition of acetylcholine release has been reported in isolated rat atria (Wetzel et al., 1985). In our study are concordant with these earlier findings: i.e., that parasympathetic actions can be regulated through an alpha-1 adrenergic receptor having different effects at pre- and postganglionic levels (Pardini et al., 1991). An alpha mediated facilitation of acetylcholine release in the perfused rat heart has also been reported (Bognar et al., 1990) and, in contrast, alpha-1 mediated inhibition of acetylcholine release has been reported in isolated rat atria (Wetzel et al., 1985). In contrast, no preganglionic alpha adrenergic effect on vagally induced bradycardia has been described in guinea pig hearts (Lew and Angus, 1983).

In our studies of repolarization, phenylephrine, alone, shortened the QT and the QTc intervals, actions blocked by WB4101 but not by CEC. There are two important aspects to this observation: first, that both the QT and the QTc, were decreased by phenylephrine is indicative of a direct alpha-1 adrenergic action on the myocardium. In contrast, that the QTc was decreased as a result of vagal stimulation whereas the absolute QT interval during ventricular pacing was not, suggests that the vagal effect on ventricular repolarization in these experiments was not direct; i.e., had there been a direct effect of right vagal stimulation on ventricular repolarization, then the paced Q-T would have shortened as well. That only the QT, decreased on vagal stimulation indicates that the slowing of sinus rate, which provides the denominator in the Bazett formula, is responsible for the decrease in the interval measured.

The effect of phenylephrine to shorten repolarization is based on an alpha adrenergic receptor subtype different from that involved in the slowing of sinus rate (blocked by CEC, not WB4101). Previous work on canine Purkinje fiber (Lee et al., 1991) and rat ventricle (Apkon and Nerbonne, 1988) has
shown an alpha-1 adrenergic receptor subtype of the same pharmacological profile (blocked by WB4101, not CEC) determines the effect of alpha adrenergic agonist on repolarization. A major difference, however, is that in dog and rat heart, alpha adrenergic agonists prolong repolarization. This prolongation is accompanied by block of I_{Ks} and/or I_{K} (Apkon and Nerbonne, 1988). The shortening of repolarization by alpha agonist in guinea pig ventricle, as recorded on ECG, is associated with a decrease in duration of the guinea pig ventricle action potential—results opposite to those in dog and rat. The effect is exerted via a PKC-dependent pathway to increase IK (Dirksen and Sheu, 1990). Hence, the effects of alpha adrenergic agonist on repolarization are clearly species dependent, involving the same alpha-1 adrenergic subtype in guinea pig, rat and dog, but different effector-coupling pathways.

That all experiments were performed in the presence of propranolol increases the likelihood that all effects were alpha adrenergic. However, it leaves unanswered the question of to what extent alpha adrenergic action and vagal interactions are expressed in the absence of beta adrenergic blockade. Wendt and Martins (1990) demonstrated that in the setting of beta adrenergic blockade, alpha adrenergic agonist increases the canine Purkinje fiber, but not the ventricular muscle effective refractory period, and the effect on Purkinje fiber is increased by acetylcholine. Kolman et al. (1976) studied vagal effects on ventricular excitability in a control setting, during left stellate stimulation and during beta adrenergic blockade. Vagal stimulation shifted ventricular strength interval curves later into diastole, signifying a prolongation of refractoriness. This effect was blocked by propranolol. Left stellate stimulation shifted the strength interval curve earlier into diastole and this action overshadowed the vagal effect when combined vagosympathetic stimulation was used. The Kolman study (1976) stresses the importance of vagosympathetic interactions and the dominant beta adrenergic component of sympathetic effect. However, it does not report any alpha adrenergic components. Moreover, it is difficult to clearly relate the results of both of these studies to our own as we measured QT and QTC intervals, whereas the earlier studies considered relative refractory periods and strength interval curves. Although we can assume a relationship between the duration of the QT interval and that of the refractory periods, the QT interval depends entirely on the duration of inward and outward currents during repolarization whereas refractory periods bring in the additional variable of the recoverability of excitability of the fast inward Na current.

In summary, in guinea pig heart, alpha adrenergic agonist, alone, slows sinus rate and its action is concordant with and additive to that of right vagal stimulation rather than resulting in accentuated antagonism. That the receptor subtype involved is alpha-1B, is suggested by the blocking effect of CEC. Consistent with observations in disaggregated guinea pig ventricular myocytes, alpha adrenergic agonist accelerates ventricular repolarization. The alpha adrenergic subtype here may be the alpha-1A or alpha-1D, as the response is blocked by WB4101 and not CEC. Although right vagal stimulation shortens the QT, this does not reflect a direct action on ventricular repolarization but rather the slowing of sinus rate. Finally, the clinical implications of this work are most readily seen in the settings of beta adrenergic blocker therapy. It is likely that in this milieu both alpha adrenergic action in their own right and interactions with the vagus will be most marked.

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