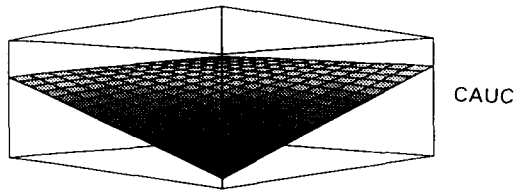

FIG. 4. Response-surface plot for ciliotoxicity of methyl and propyl hydroxybenzoates. CAUC is the complement of the area under the curve (% s).

demonstrating visually why a quadratic model provides a better description of the response than does a model which includes only linear terms.

The surface-response study provides a means for quantitatively defining the range of acceptable concentrations of the two alkyl hydroxybenzoates for preserving nasal formulations. In practice the combination with the highest preservative concentrations consistent with absence of ciliotoxicity would be chosen. On intra-nasal application, dilution with nasal secretions would reduce the preservative concentrations and hence reduce ciliotoxicity. Our results suggest that a combination of the data reported here with data on the antimicrobial kinetics of the two compounds would enable optimization of their use with respect to both efficacy and toxicity.

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The purely chronotropic effects of relaxin in the rat isolated heart

G. ROGER THOMAS, RICHARD VANDLEN*, *Departments of Cardiovascular Research and *Protein Chemistry, Genentech Inc., 460 Point San Bruno Boulevard, South San Francisco, CA 94080, USA*

Abstract—The endogenously occurring protein relaxin was evaluated for its cardiac effects in the rat isolated heart. Relaxin caused a dose-dependent tachycardia without positive inotropic effects in intact preparations and in preparations where the atria had been removed. It is concluded that relaxin acts on both the atrial and ventricular pacemakers to increase heart rate, but the precise mechanism of action remains unknown.

Binding sites for relaxin, a heterodimeric member of the insulin family of proteins, have been identified in the rat atrium (Osheroff et al 1992). Following these observations, subsequent studies have shown that relaxin can induce both inotropic and chronotropic effects on rat isolated atrial preparations (Kakouris et al 1992; Ward et al 1992). However, it is not clear whether relaxin has effects on other excitable cardiac tissue. This study was conducted to elucidate further the cardiac effects of relaxin. This was accomplished using isolated hearts where the effects on atrial and ventricular pacemaker tissue could be studied separately.

Materials and methods

Male Sprague-Dawley rats, 300–350 g (Charles River, Portage, IN, USA), were anaesthetized with 60 mg kg^{-1} intraperitoneal pentobarbitone sodium (Fort Dodge Laboratories Inc., Fort Dodge, IA, USA). Following thoracotomy the hearts were removed and placed in 4°C Krebs–Henseleit solution, containing (mM): NaCl 118, KCl 4·7, $CaCl_2$ 2.5, $MgSO_4$ 1.6, $NaHCO_3$ 25, KH_2PO_4 1·2 and glucose 11, and gassed with 95% O_2–5% CO_2. Any associated lung and thymic tissue was trimmed and the ascending aorta cannulated. The heart was then perfused at 10 mL min^{-1} with Krebs–Henseleit solution at 37°C. A small incision was made in the left atrium and a saline-filled balloon (Hugo Sachs Elektronik, March-Hugstetten, Germany) connected to a pressure transducer (Gould Electronics, Valley View, OH, USA) was placed into the ventricle for the measurement of left ventricular developed pressure (LVDP). LVDP was taken as the maximal pulse pressure in mmHg developed during each systole. From this pressure recording, heart rate was electronically derived on a Grass polygraph (Quincy, MA, USA).

In six experiments, after a 15–20 min stabilization period, the atria were removed. The heart rate was then allowed to stabilize at its new rate before the administration of relaxin. In all other experiments the atria were left intact and drugs administered after a 20-min stabilization period.

Relaxin was administered as 50-μL bolus injections into the perfusion fluid immediately before it entered the heart. Cumulative dose-response curves were constructed using human recombinant gene-2 relaxin (Genentech Inc., South San Francisco, CA, USA). Adrenaline bitartrate (Sigma Chemical Co., St Louis, MO, USA) was dissolved as 1 mg mL^{-1} stock solution in 0·9% NaCl (saline) containing 1 mg mL^{-1} sodium ascorbate and administered into the perfusate as 50- or 150-μL bolus injections. Serial dilutions of both drugs were made using saline. All doses were given at 15-min intervals. Relaxin and adrenaline were tested in separate preparations.

Statistical significance was determined by a two-tailed Student's unpaired t-test and $P < 0.05$ taken as significant.

Correspondence: G. R. Thomas, Cardiovascular Research Department, Genentech Inc., 460 Point San Bruno Boulevard, South San Francisco, CA 94080, USA.

Results

Rat isolated heart preparations had a basal beating rate of 270 ± 7 beats min^{-1} and an LVDP of 83 ± 4 mmHg. After removal of the atria, heart rate fell to 139 ± 15 beats min^{-1} ($P < 0.05$) while LVDP increased to 143 ± 9 mmHg ($P < 0.05$).

The addition of relaxin to the perfusion fluid of intact heart preparations caused a dose-dependent chronotropic response reaching a maximum beating rate of 412 ± 18 beats min^{-1} at 83 pM ($P < 0.05$) (Fig. 1). In preparations where the atria were removed, the addition of relaxin also caused a dose-dependent chronotropic effect with a maximum rate of 268 ± 7 beats min^{-1} in response to 83 pM ($P < 0.05$) (Fig. 1). These effects lasted up to 2 h after a single bolus administration of 830 pM. Conversely, a short-lasting dose-dependent chronotropic effect was also observed in response to adrenaline administration. The maximal attainable beating rate following adrenaline was 349 ± 8 beats min^{-1} at 150 pM ($P < 0.05$) (Fig. 1). Higher doses caused excessive arrhythmia where precise measurement of beating rate was difficult.

Concomitant with the chronotropic response to adrenaline was a dose-dependent positive inotropic effect. LVDP increased to 195 ± 10 mmHg at 4·5 pM ($P < 0.05$). Higher doses resulted in slightly attenuated positive inotropic responses (Fig. 1). Unlike adrenaline, relaxin had no positive inotropic effects. In those preparations where the beating rate was reduced by removing the atria and having higher LVDP, relaxin (0·83 pM) significantly reduced the LVDP to control levels ($P < 0.05$) (Fig. 1).

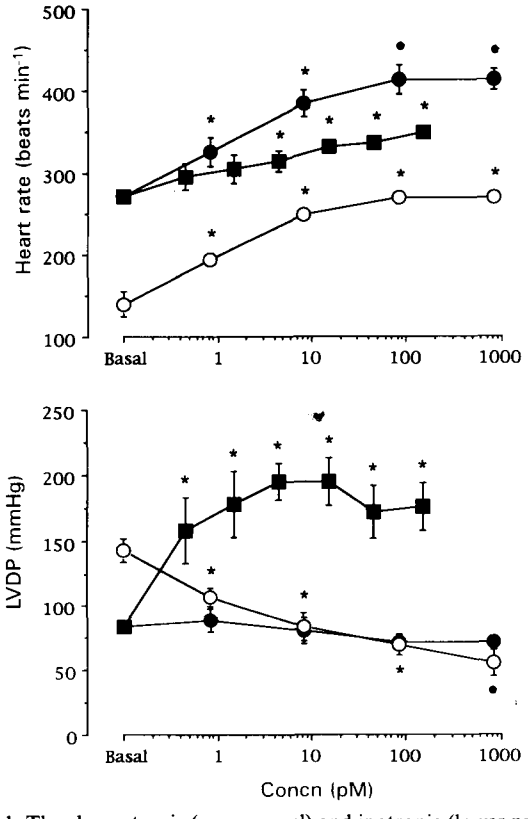

FIG. 1. The chronotropic (upper panel) and inotropic (lower panel) effects of recombinant human relaxin in intact rat isolated heart preparations (●) (n = 4) and in preparations where the atria were eliminated (○) (n = 6). These are compared with the effects of adrenaline (■) (n = 6) in intact preparations. Each point represents the mean ± s.e.m. Asterisks indicate statistical significance between each point and its respective basal rate. LVDP = left ventricular developed pressure.

Discussion

Previous studies have shown that relaxin has positive chronotropic and inotropic effects on rat isolated atrial preparations (Kakouris et al 1992; Ward et al 1992). However, in the isolated heart preparation, relaxin is purely a chronotropic agent. These disparate results may indicate subsets of the relaxin receptor found by Osheroff et al (1992), or merely a lack of the inotropic response to the receptor stimulation in ventricular tissue. Although Osheroff et al (1992) did not see relaxin binding sites in the rat ventricle, no systematic search for receptors in the AV node and the conduction system has been carried out to date. In this study it was apparent that the relaxin-induced tachycardia response is not confined to the atria (presumably the SA node) but was also present in ventricular pacemaker tissue (AV node or the bundle of His). It was also noticeable that the chronotropic effect reached a maximum in both the atrial- and ventricular-driven preparations. In fact the dose-response curves appear parallel. The precise mechanism of action for the cardiac effects of relaxin remain unknown, but previous studies have shown that this protein can increase cyclic AMP in human endometrial (Fei et al 1990) and endometrial glandular epithelial cells (Chen et al 1988) as well as in rat (Hsu et al 1985) and rhesus monkey (Kramer et al 1990) myometrial cells. However, it is unlikely that this single mechanism of action for relaxin accounts for all of the cardiac responses since β_1-adrenoceptor agonists activate adenylate cyclase. This results in increased transmembrane Ca^{2+} influx, increased capacity and rate of Ca^{2+} accumulation by the sarcoplasmic reticulum and enhanced sensitivity of troponin-C to Ca^{2+} (Oye 1975). The culmination of all of these effects, as shown here, are tachycardia and an even more pronounced positive inotropic effect.

During the course of the study we also observed that in isolated heart preparations where the atria were removed, the beating rate was, as expected, halved. Concomitant with this bradycardia was an increase in the LVDP, probably due to a shift in the energy utilization of the muscle. Relaxin-induced restoration of ventricular preparations' beating rate to that of the intact heart, at 8·3 pM or above, reverses this bradycardia-associated inotropy. This suggests that the observation was a reversal of a bradycardia-induced inotropy rather than a direct negative inotropic effect of relaxin, since there was no attenuation of LVDP seen in the intact preparations.

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