Br. J. Pharmac. (1971), **42**, 364–370.

Effects of isoprenaline and aminophylline on the chronotropic responses of the isolated guinea-pig heart to vagal stimulation and acetylcholine

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Summary

1. In isolated perfused guinea-pig hearts, isoprenaline (2×10^{-8} g/ml) reduced the negative chronotropic responses to vagal stimulation and to exogenous acetylcholine.

2. The β-adrenoceptor antagonist LB46 (2×10^{-8} g/ml) abolished the effect of isoprenaline in reducing vagal bradycardia. LB46 itself did not alter the responses to vagal stimulation.

3. Increase in ^{86}Rb efflux induced by acetylcholine was not affected by isoprenaline.

4. Aminophylline ($2{\cdot}3 \times 10^{-4}$ g/ml) almost abolished the negative chronotropic effect of vagal stimulation.

5. The 'antivagal' effects of isoprenaline and aminophylline may be at a common site beyond the level of the cardiac β-adrenoceptors, perhaps related to cyclic-3′,5′-AMP and/or sodium transport.

Introduction

There is considerable evidence of an interaction between sympathetic and parasympathetic systems on heart rate (Lynch & Essex, 1956; Leaders & Long, 1962; Leaders, 1963; Miller, Pendleton & Richmond, 1968; Vassale, Mandel & Holder, 1970), though others have questioned this (Misu & Kirpekar, 1968; Warner & Russell, 1969).

My experiments were undertaken to find out whether such an interaction occurred at the level of the cholinoceptor or beyond, by studying the effect of isoprenaline and aminophylline on the responses of the isolated atrium to cholinergic stimuli. Some of these observations have been demonstrated to the British Pharmacological Society (Hadhazy, 1970).

Methods

Guinea-pigs weighing 500–600 g were anaesthetized by 1·5 g/kg urethane, intraperitoneally. The trachea was cannulated and the animals were artificially

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ventilated. The right vagus nerve was dissected up to the aortic arch. Five hundred units of heparin were injected into the left jugular vein. The aorta was cannulated *in situ* and perfused with oxygenated (95% O_2–5% CO_2) McEwen's solution at 29° C (McEwen, 1956). The heart was removed with the vagus nerve attached together with a part of the trachea and oesophagus and perfusion continued at a constant rate of 7 ml/minute. The nerve was placed on a pair of platinum electrodes and stimulated supramaximally with rectangular pulses of 1 ms duration at increasing frequencies for 20 or 30 seconds. The heart rate was measured from the electrical activity of the sinus node recorded with electrodes attached to the left auricle. In the experiments on the effect of exogenous ACh, the same preparation without the vagus nerve was used. ACh was injected into the perfusion cannula in 0·2 ml of McEwen's solution. The other drugs were added to the reservoir containing the perfusion fluid. Heart rate was measured during a period of 10 s after the injection of ACh or 30 s after commencement of vagal stimulation and compared with the control period.

^{86}Rb efflux

Isolated left atria were incubated for 1 h in Krebs solution containing 5μCi ^{86}Rb/ml (as ^{86}RbCl, 1 mM). Subsequent efflux of ^{86}Rb into Krebs solution containing 1 mM unlabelled RbCl was measured by Cerenkov counting using a liquid scintillation spectrometer. The atria were stimulated at 2 Hz throughout the experiment except where indicated.

Statistical significance was calculated by Student's *t* test and parallel regression line analysis (Snedecor, 1956).

The following drugs were used: acetylcholine chloride (B.D.H.); isoprenaline sulphate (Martindale Samoore); aminophylline (Martindale Samoore); (±)-4-(2-hydroxy-3-isopropylaminopropoxy)-indole (LB46) kindly supplied by Dr. K. Saameli and Dr. M. Taeschler (Sandoz Ltd., Basle, Switzerland); ^{86}RbCl (Radiochemical Centre, Amersham), specific activity: 2·4 mCi/mg. All doses and concentrations are given in terms of the salts.

Results

Chronotropic responses to vagal stimulation

Isoprenaline (2×10^{-8} g/ml) reduced the magnitude of the negative chronotropic response to stimulation of the vagus nerve at 1–20 Hz (Fig. 1.). The slope of the frequency-response curve was reduced, but the percentage reduction of the effect of vagal stimulation by isoprenaline was approximately the same at all frequencies. The maximum effect of vagal stimulation was depressed by about 50%. These effects of isoprenline were fully prevented by adding a β-adrenoceptor antagonist (LB46) to the perfusion fluid; LB46 itself had no effect on the response to vagal stimulation.

Chronotropic responses to acetylcholine

Isoprenaline (2×10^{-8} g/ml) reduced both the magnitude and duration of the bradycardia produced by acetylcholine (Fig. 2). The effect of isoprenaline on the relation between the 'peak' inhibition and the dose of acetylcholine is shown in Fig. 3. Isoprenaline did not modify the slope of the curve, nor reduce the maximum bradycardia produced by acetylcholine.

^{86}Rb efflux

One possible explanation of the above effects is that isoprenaline might modify the action of acetylcholine on the cardiac cell membrane. The primary effect of acetylcholine on the membrane is to increase K^+ conductance (Hutter, 1961). In practice, fluxes of ^{86}Rb produce a convenient measure of changes in K^+ conductance in this tissue (van Zwieten, 1968) so that measurements of ^{86}Rb efflux yield a more direct index of the action of acetylcholine on the cell membrane than measurement of heart rate alone.

The interactions of acetylcholine and isoprenaline on the rate of efflux of ^{86}Rb from isolated atria previously incubated in $^{86}RbCl$ was studied in three experiments. As shown in Fig. 4., acetylcholine increased the rate of efflux of ^{86}Rb from the isolated atrium. This increased efflux was similar both when the heart was stimulated and at rest, and hence was not due to changes in heart rate. Addition of isoprenaline produced a very slight increase in ^{86}Rb efflux but did not modify the effect of acetylcholine.

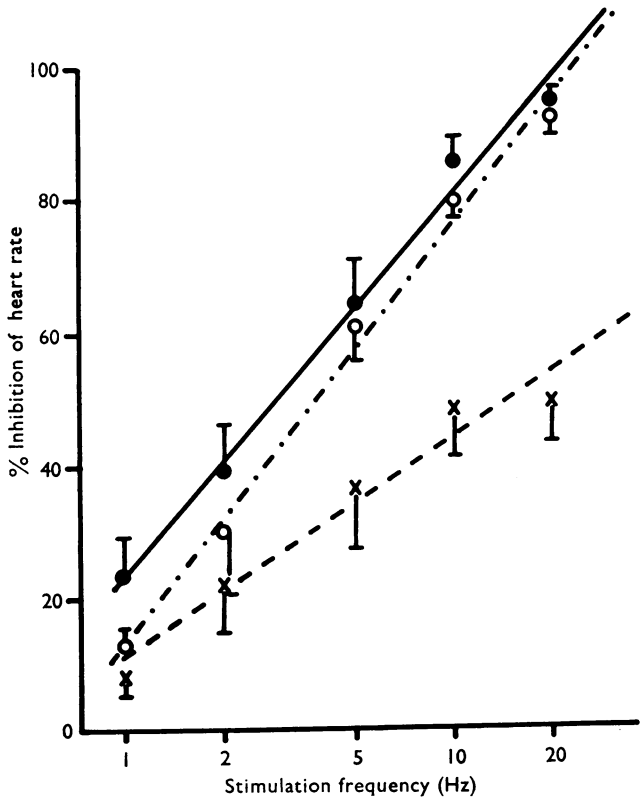

FIG. 1. Effect of isoprenaline on the negative chronotropic response to vagal stimulation. Abscissa, nerve stimulation frequency; ordinate, percentage inhibition of heart rate. 1. (●——●), Control effect of vagal stimulation; $y=23{\cdot}2+58{\cdot}1$ x; $R=0{\cdot}89$; $n=35$. 2. (×----×), Vagal stimulation in the presence of isoprenaline (2×10^{-8} g/ml); $y=10{\cdot}8+33{\cdot}4$ x; $R=0{\cdot}67$; $n=35$. 3. (○-·-·-○), Vagal stimulation in the presence of isoprenaline (2×10^{-8} g/ml)+LB46 (2×10^{-8} g/ml); $y=12{\cdot}9+64{\cdot}1$ x; $R=0{\cdot}94$; $n=25$. Vertical bars: ±S.E. Values of P for the slopes; 1 versus 2 $P<0{\cdot}005$; 1 versus 3 $P<0{\cdot}4$; 2 versus 3 $P<0{\cdot}001$. The inhibitory effect of isoprenaline at 1 and 2 Hz is statistically significant ($P<0{\cdot}001$).

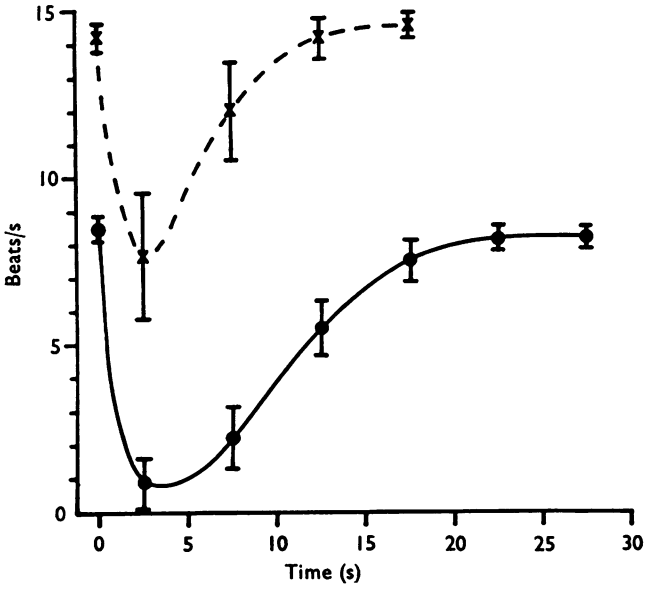

FIG. 2. Effect of isoprenaline on the duration of action of ACh. Abscissa, time after the injection of ACh; ordinate, heart beats/second. (●——●), Control effect of ACh; time from peak to half recovery: 11 seconds. (×----×), Effect of ACh in the presence of isoprenaline (2×10^{-8} g/ml); time from peak to half recovery: 6 s, $n=6$. Vertical bars: ±S.E.

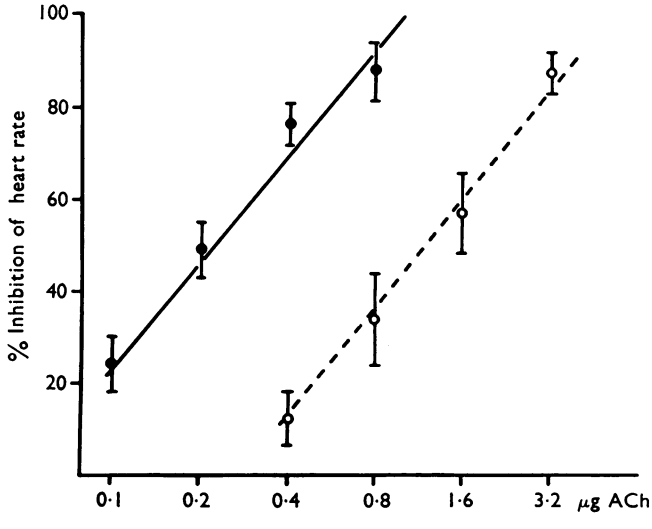

FIG. 3. Effect of isoprenaline (2×10^{-8} g/ml) on the negative chronotropic response to injected ACh. Abscissa, doses of ACh; ordinate, percentage inhibition of heart rate. (●——●), Control effect of ACh; $y=98{\cdot}7+71{\cdot}9\,x$; $R=0{\cdot}87$; $n=24$. (○- - -○), Effect of ACh in the presence of isoprenaline (2×10^{-8} g/ml); $y=43{\cdot}6+82{\cdot}2\,x$; $R=0{\cdot}85$; $n=24$. Vertical bars: ±S.E.

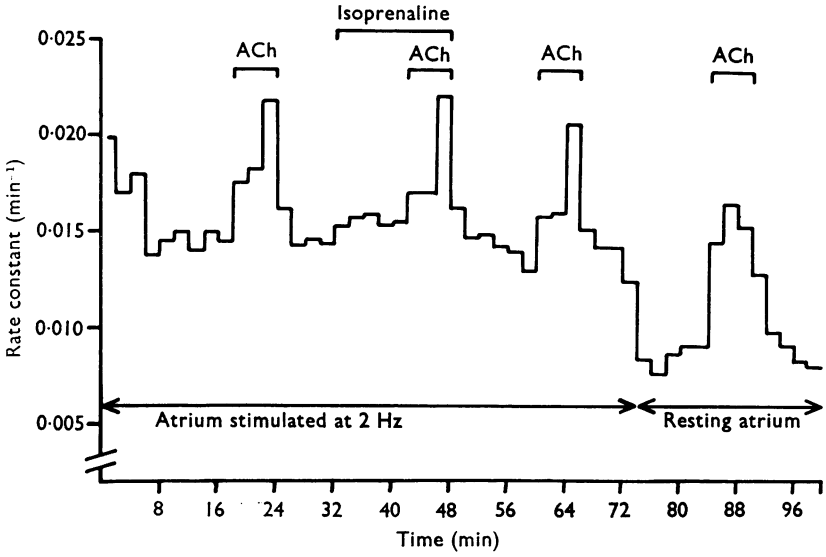

FIG. 4. Effects of ACh (10^{-6} g/ml) and isoprenaline on the ^{86}Rb efflux from the isolated left guinea-pig atrium. Abscissa, time; ordinate, rate constant. Note that addition of isoprenaline did not affect the action of ACh on ^{86}Rb efflux.

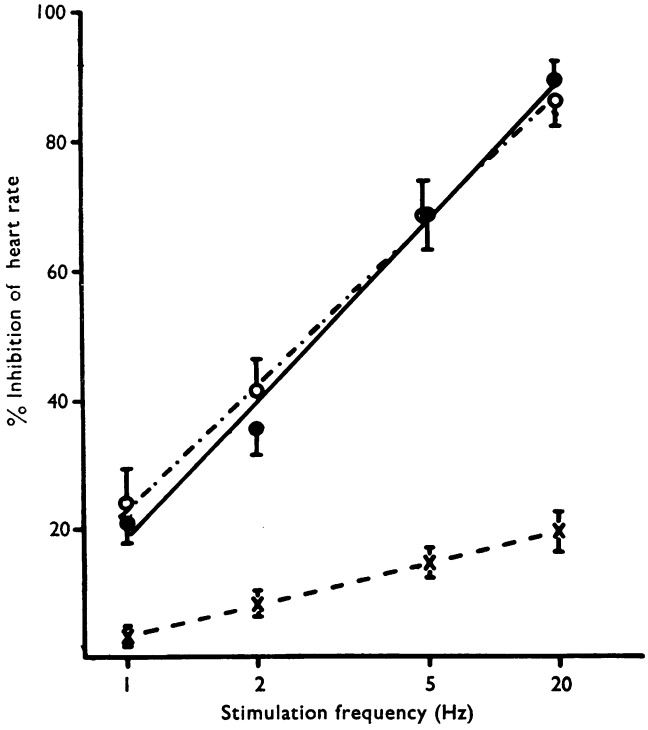

FIG. 5. Effect of aminophylline ($2{\cdot}3 \times 10^{-4}$ g/ml) on vagal bradycardia. Abscissa, nerve stimulation frequency (Hz); ordinate, percentage reduction of heart rate. (●——●), Control effect of vagal stimulation; $y=71{\cdot}4 \times +18{\cdot}4$; $R=0{\cdot}93$. (×----×), Vagal stimulation in the presence of aminophylline; $y=16{\cdot}4 \times +3{\cdot}1$; $R=0{\cdot}77$. (○-·-·-○), Recovery; $y=63{\cdot}2 \times +23{\cdot}5$; $R=0{\cdot}90$, $n=6$. Vertical bars: ±S.E.

Effect of aminophylline

Since isoprenaline did not appear to modify the direct action of acetylcholine upon the membrane, an effect beyond the membrane was likely. One possibility is that the action of isoprenaline is mediated through its stimulatory effect on cyclic 3′5′-AMP (Murad, Chi, Rall & Sutherland, 1962). Aminophylline also elevates cyclic AMP levels, by inhibiting phosphodiesterase (Butcher & Sutherland, 1962) so that the effect of aminophylline on the chronotropic action of vagal stimulation seemed worth studying.

The effect of aminophylline was investigated in six isolated perfused guinea-pig heart-vagus nerve preparations. Aminophylline ($2{\cdot}3 \times 10^{-4}$ g/ml) almost abolished the response to vagal stimulation, in a manner resembling that of isoprenaline (Figs 5 and 1). This effect was fully reversible on reverting to perfusion with normal McEwen's solution.

Discussion

Isoprenaline reduced the chronotropic effects of both vagal stimulation and acetylcholine. This was prevented by β-adrenoceptor blockade, and hence resulted from β-adrenoceptor stimulation.

The effects of isoprenaline on the two forms of cholinoceptor activation differed in that the maximum response to vagal stimulation was depressed whereas the dose-response curve to acetylcholine was shifted in a parallel manner with no reduction of maximum. This difference may result from a failure of the heart to respond to increasing frequencies of vagal stimulation above 20 Hz—perhaps because of a ceiling to acetylcholine release. Although no direct information on this aspect of vagal action is available, the sympathetic ganglia (and presumably parasympathetic ganglia) do not readily transmit impulses at frequencies above 20 Hz.

It is also possible that isoprenaline reduced transmitter release from the vagus nerve, though other reports indicate that adrenergic depression of acetylcholine release from parasympathetic nerves occurs with α- but not β-adrenoceptor stimulants (Vizi, 1968 ; Paton & Vizi, 1969). Certainly, the reduction of acetylcholine-induced bradycardia is clearly indicative of a postjunctional effect and, in the absence of contrary evidence, it seems most reasonable to ascribe the antivagal action of isoprenaline to a postjunctional action.

A direct interaction with the acetylcholine receptor, or a change in membrane K^+ permeability can probably be excluded, since isoprenaline did not materially affect the action of acetylcholine on the efflux of ^{86}Rb. On the other hand, the rate of rise of the pacemaker potential—and hence the heart rate—is probably determined by the balance between membrane K^+ and Na^+ conductance (Trautwein, 1963). In isolated rabbit and guinea-pig atria-vagus nerve preparations, an increase of extracellular K^+ or reduction of Na^+ enhanced the negative chronotropic response to vagal stimulation (Toda, Fujiwara & Shimamoto, 1964 ; Toda & West, 1967). Since no measurements of Na^+ movements were made, an effect on Na^+ cannot be excluded.

Perhaps the most likely mechanism of action of isoprenaline is through its effect on cellular concentrations of cyclic-3′5′-AMP. This view is prompted by the similar and very striking antivagal action of aminophylline, and is perhaps supported by

the finding of Levine & Vogel (1966) that injection of cyclic AMP produced tachycardia in dogs. It would be interesting to know whether this compound, or other agents which raise cardiac cyclic AMP concentrations (such as glucagon), also inhibit vagal bradycardia.

I am most grateful to Dr. D. F. J. Mason, Dr. D. A. Brown and Mr. C. N. Scholfield for their useful suggestions and help. This work was supported by a grant from the International Atomic Energy Agency, Vienna, of which I am a fellow.

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(*Received December* 31, 1970)