Br. J. Pharmac. (1973), **48**, 620–628.

Anaphylaxis in the guinea-pig isolated heart: selective inhibition by burimamide of the positive inotropic and chronotropic effects of released histamine

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Summary

1. Anaphylaxis was induced in the isolated heart of the guinea-pig in the presence of burimamide at concentrations of 4×10^{-5}M and $2{\cdot}7\times10^{-4}$M.

2. Burimamide did not affect the immunological release of histamine; however, it selectively antagonized the positive inotropic and chronotropic effects of released histamine. The antagonism of the positive chronotropic effect was concentration-dependent.

3. Neither the negative dromotropic effect nor the decrease in coronary flow rate occurring during anaphylaxis were inhibited by burimamide.

4. The results are in agreement with the double histamine receptor theory and suggest that, in the heart of the guinea-pig, H_2-receptors are involved in the positive inotropic and chronotropic effects of released histamine, and H_1-receptors in the negative dromotropic effect.

Introduction

Sensitized guinea-pig hearts respond to antigen *in vitro* with a reaction characterized by sinus tachycardia, atrioventricular block, decrease in coronary flow and brief stimulation of ventricular contraction followed by failure. During cardiac anaphylaxis, histamine is released into the coronary perfusate (Feigen & Prager, 1969). A quantitative comparison between the cardiac effects of exogenous and immunologically released histamine reveals that most of the changes occurring during cardiac anaphylaxis are mediated by histamine (Levi, 1972).

Compounds such as theophylline, glucagon and the dibutyryl derivative of cyclic adenosine monophosphate have been shown to interfere with the immunological release of histamine during cardiac anaphylaxis (Levi, 1970; Capurro & Levi, 1971; Levi, 1971). Although these compounds diminish the amounts of histamine released, they all display a considerable direct cardiostimulatory action which adds to the cardiostimulatory action of histamine. Attempts to prevent the anaphylactic reaction of the guinea-pig isolated heart with mepyramine have failed (Hahn & Bernauer, 1970), probably because 'classical' antihistamines do not antagonize the positive chronotropic and inotropic effects of histamine (Trendelenburg, 1960; Bartlet, 1963).

Recently Black and co-workers have developed a new antihistamine, burimamide, which is capable of inhibiting the histamine-induced stimulation of the guinea-pig atrium (Black, Duncan, Durant, Ganellin & Parsons, 1972).

The purpose of our investigation was to study the effects of burimamide on the anaphylaxis of the isolated heart.

Methods

Male Camm-Hartley guinea-pigs weighing between 250 and 300 g were sensitized by two intraperitoneal injections of 10 mg ovalbumin (grade V, crystalline egg albumin, Sigma Chemical Co., St. Louis, Missouri) on two consecutive days (Feigen, Vaughan-Williams, Peterson & Nielsen, 1960). Fifteen to thirty days after sensitization the animals were stunned by a blow to the base of the skull. The heart was removed and mounted via an aortic cannula in a modified Langendorff apparatus (Beani, 1953) and perfused at a constant pressure (40 cm of water) with oxygenated Ringer solution at 37·5° C. The composition of the Ringer solution was (mM): Na^+, 160; Cl^-, 164; K^+, 5·6; Ca^{++}, 2·2; HCO_3^-, 5·9; glucose 5·5. Hearts were perfused for 45 min to 1 h before experimentation was begun, by which time heart rate and contraction had reached a steady state.

The apex of the heart was connected by a nylon thread to a force-displacement transducer (model FTO3B, Grass Instruments, Quincy, Massachusetts). Isometric ventricular contractions were recorded on one channel of a two-pen oscillograph (model P2, Texas Instruments, Inc., Digital System Division, Houston, Texas) at paper speeds ranging between 1 and 250 mm/s, and on one channel of another two-pen recorder (Dynograph model 542, Offner Electronics, Inc., Schiller Park, Illinois) at a constant paper speed of 0·25 mm/second.

Surface electrocardiograms were differentially recorded between the right atrium and the left ventricle. The electrocardiograms were displayed on the second channel of the Texas Instruments oscillograph. A level discriminator was adjusted to be triggered by the QRS complex. Its pulse output was connected to a cardiotachometer (model 370, Harvard Apparatus Co., Millis, Massachusetts). Cardiotachometer output (heart rate) was displayed on the second channel of the Offner recorder. Coronary perfusate was collected over intervals ranging from 1 to 5 min; the volume of perfusate was recorded.

Antigenic challenge was accomplished by rapid intra-aortic injection of 1 mg of ovalbumin dissolved in a constant volume of warm oxygenated Ringer solution. A sample of coronary perfusate was collected for 2 min before the challenge with antigen. Six 2-min post-challenge samples were also collected.

In some experiments hearts were subjected to anaphylaxis in the presence of burimamide (*N*-methyl-*N'*[4-(4(5)-imidazolyl)butyl] thiourea), kindly supplied by Dr. J. W. Black (Smith, Kline & French Laboratories, Welwyn Garden City, Hertfordshire). Six hearts were perfused with burimamide at a concentration of 4×10^{-5}M and six other hearts at $2{\cdot}7\times10^{-4}$M. These concentrations were shown by Black *et al.* (1972) to produce a 15- and a 100-fold increase respectively in the ED_{50} of histamine (positive chronotropic effect in the isolated guinea-pig atria). Hearts were continuously perfused with burimamide beginning 10 min before and ending 20 min after antigenic challenge.

Histamine was determined in the coronary perfusate by the fluorometric procedure of Anton & Sayre (1969), and an Aminco-Bowman spectrophotofluorometer was used (American Instruments Co., Inc., Silver Spring, Maryland). A 5-point standard calibration curve for histamine base from 0·02 to 6 μg/ml was determined

for each assay. Burimamide in concentrations up to $2{\cdot}7\times10^{-4}$M did not interfere with the assay.

In order to determine the effect of burimamide on the positive chronotropic effect of histamine, concentration-effect curves were established for histamine (histamine dihydrochloride, Sigma Chemical Company; all concentrations of histamine refer to the free base) and histamine plus burimamide at 4×10^{-5}M and $2{\cdot}7\times10^{-4}$M. Each heart was perfused for 10 min with only one concentration of either histamine or histamine plus burimamide.

Results

Effects of antigenic challenge

In sensitized hearts the intra-aortic injection of antigen resulted in a crisis of cardiac function. Antigenic challenge induced an abrupt increase in the amplitude of ventricular contraction (Figure 1A). The positive inotropic effect was short-

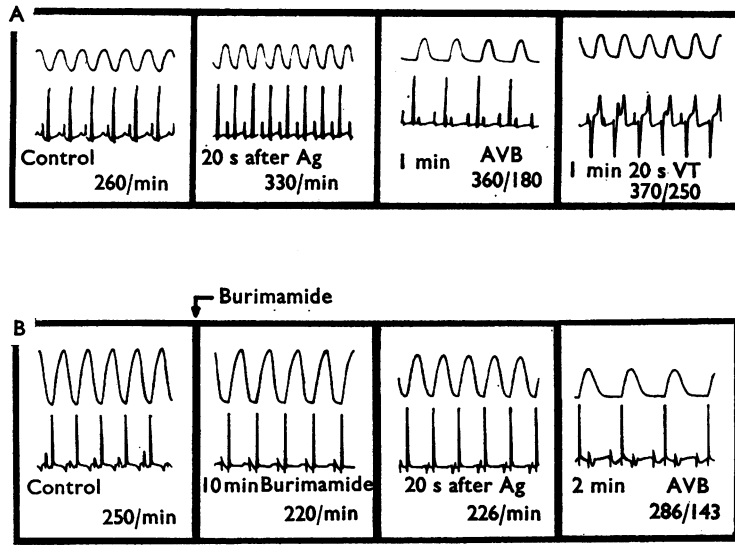

FIG. 1. Recordings of isometric ventricular contraction (upper tracing) and surface electrocardiogram (lower tracing) during isolated heart anaphylaxis (A) and isolated heart anaphylaxis in presence of burimamide $2{\cdot}7\times10^{-4}$M (B). The first panel of A is the control immediately preceding the injection of antigen. In B the first panel is the control preceding burimamide perfusion; the second panel, at 10 min of burimamide perfusion, is immediately followed by antigen injection. Time of recording in min from antigen injection is shown at the lower left corner of each panel. Numbers at the bottom right of each panel refer to heart rates; both atrial and ventricular rates are shown when in the presence of atrioventricular conduction block (Ag=antigen, AVB=atrioventricular block, VT=ventricular tachycardia).

lasting and was followed by a negative inotropic effect. Also, the sinus rate increased at 15 s and reached a peak at about 2 minutes. The sinus rate gradually declined over the next 28 min (Figure 2B). Atrioventricular conduction was progressively impaired until atrioventricular block developed, approximately 40 s after challenge (Figure 2C). Conduction arrhythmias were invariably present and lasted about 9 minutes. Subsequently, atrioventricular conduction progressively improved (Fig. 2C and Table 1). Multifocal ventricular extrasystoles and ventricular tachycardia occurred frequently. During cardiac anaphylaxis, the rate of

TABLE 1. *Conduction arrhythmias during cardiac anaphylaxis.*

Concentration of burimamide	Incidence	Onset (s±S.E.)	Duration (min±S.E.)
0	14/14	42	8·87
(n=14)		±3	±1·25
4×10^{-5}M	6/6	40	6·72
(n=6)		±3	±1·83
$2{\cdot}7\times10^{-4}$M	5/6	69*	5·47**
(n=6)		±10	±0·87

*Significantly different ($P<0{\cdot}005$) from no drug. **$0{\cdot}1>P>0{\cdot}05$.

TABLE 2. *Coronary flow rate during cardiac anaphylaxis*

Concentration of burimamide	Coronary flow rate		
	Before antigen (ml/min±S.E.)	Anaphylaxis (ml/min±S.E.)	% Change
0	5·2	3·9*	−25·0
(n=14)	±0.3	±0.3	
4×10^{-5}M	5·8	4·1*	−29·3**
(n=5)	±0·5	±0.3	
$2{\cdot}7\times10^{-4}$M	4·7	3·2*	−31·9**
(n=6)	±0·3	±0·2	

*Significantly different ($P<0{\cdot}025$) from own controls. **Not significantly different from change without drug.

TABLE 3. *Histamine release during cardiac anaphylaxis.*

Concentration of burimamide	Histamine release (μg/g±S.E.)
0	3·13
(n=14)	±0·25
4×10^{-5}M	2·88
(n=6)	±0·17
$2{\cdot}7\times10^{-4}$M	3·25
(n=6)	±0·25

coronary flow decreased by 25% (Table 2). All of these changes were accompanied by the release of histamine into the coronary perfusate (Table 3). The release of histamine reached a peak at 2 min and ceased about 10 min after challenge (Figure 2A). The increase in sinus rate was correlated with the rate of histamine release into the coronary perfusate (Figure 3).

Effects of burimamide

Perfusion of the heart with burimamide at 4×10^{-5}M did not alter the parameters of cardiac function (Table 4). Perfusion with $2{\cdot}7\times10^{-4}$M moderately reduced sinus rate, whereas other parameters were unchanged (Table 4).

TABLE 4. *Effects of burimamide on certain parameters of cardiac function.*

	Control	Burimamide 4×10^{-5}M (n=6)	Control	Burimamide $2{\cdot}7\times10^{-4}$M (n=6)
Contraction (g±S.E.)	8·2 ±0·6	8·8 ±0·6	6·6 ±0·5	6·6 ±0·5
Rate (beats/min±S.E.)	252 ±5	251 ±5	243 ±6	221* ±6
P-R interval (ms±S.E.)	62 ±2	63 ±2	58 ±2	59 ±2
Coronary flow rate (ml/min±S.E.)	5·9 ±0·3	5·8 ±0·5	4·5 ±0·3	4·7 ±0·3

*Significantly different ($P<0{\cdot}025$) from own control.

Effects of antigenic challenge in the presence of burimamide

In sensitized hearts continuously perfused with burimamide at either concentration, the injection of antigen resulted in a prolonged negative inotropic effect. This effect was not preceded by the transient positive inotropic effect which characteristically followed antigenic challenge in untreated hearts. At a concentration of 4×10^{-5}M, burimamide slightly reduced the peak positive chronotropic effect of anaphylaxis, while at a burimamide concentration of $2{\cdot}7\times10^{-4}$M, the peak positive chronotropic effect was greatly reduced (Figures 1B and 2B).

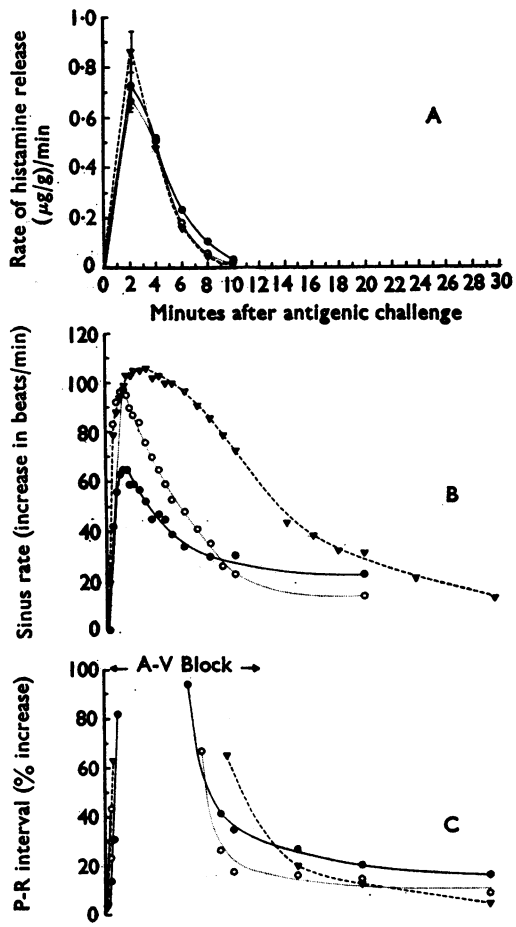

FIG. 2. Time-courses of (A) rate of histamine release, (B) increase in sinus rate and (C) prolongation of the P–R interval during isolated heart anaphylaxis . ▽---▽, isolated heart anaphylaxis (untreated) (n=14); ○- - -○, isolated heart anaphylaxis in the presence of burimamide 4×10^{-5}M (n=6); ●——●, isolated heart anaphylaxis in the presence of burimamide $2{\cdot}7\times10^{-4}$M (n=6). Abscissae: time from injection of antigen. Ordinates: in A, average histamine release (vertical bars=S.E.) measured over 2-min intervals; in B, sinus rate increase from values immediately preceding injection of antigen; in C, prolongation of P–R interval from values immediately preceding injection of antigen (these curves are interrupted during conduction arrhythmia). Average control sinus rates are the same as in Figure 3. Average control (±S.E.) P–R intervals were 61 (±1) for ▽---▽, 62 (±2) for ○- - -○, and 58 (±2) for ●——●.

The impairment of atrioventricular conduction occurring during anaphylaxis was not inhibited by burimamide (Figures 2C and 1B). At a concentration of 4×10^{-5}M, conduction arrhythmias did not differ in incidence, onset or duration from arrhythmias occurring during anaphylaxis in the absence of burmamide (Table 1). At the concentration of $2{\cdot}7\times10^{-4}$M, arrhythmias occurred less frequently and were significantly delayed in their onset; their duration was shorter but did not quite

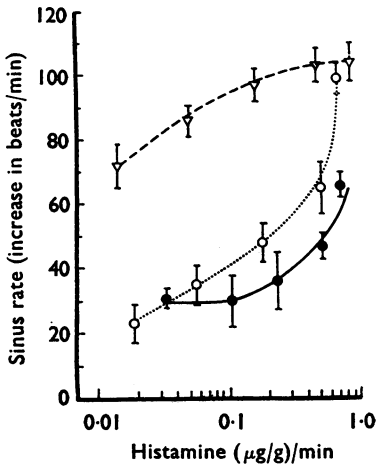

FIG. 3. Correlation between histamine release and increase in sinus rate. Effect of burimamide ▽---▽, isolated heart anaphylaxis (untreated) (n=14); ○---○, isolated heart anaphylaxis in the presence of burimamide 4×10^{-5}M (n=6); ●——●, isolated heart anaphylaxis in the presence of burimamide $2{\cdot}7\times10^{-4}$M (n=6). Abscissae: average histamine release measured over 2 min intervals for 10 min following antigen. Ordinates: maximum sinus rate increase (vertical bars=S.E.) over the same 2 min intervals, from values immediately preceding antigen injection. The curves were fitted by eye. Average (±S.E.) control sinus rates were 244 (±6) for ▽---▽, 251 (±5) for ○---○, and 222 (±6) for ●——●.

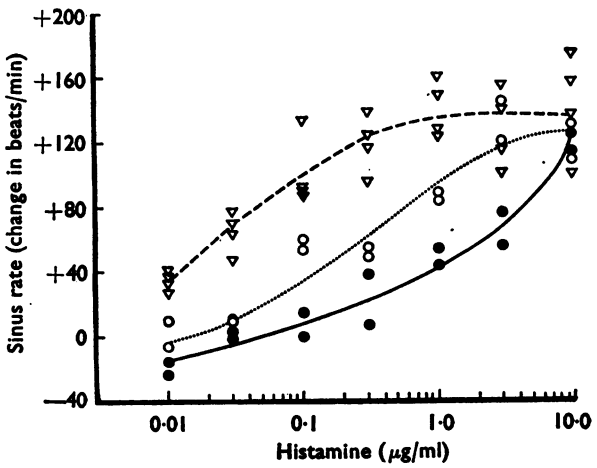

FIG. 4. Antagonism by burimamide of the positive chronotropic effect of exogenous histamine in isolated hearts. Hearts were continuously perfused with histamine alone, ▽---▽, in 28 experiments; together with burimamide 4×10^{-5}M, ○---○, in 14 experiments; or together with burimamide $2{\cdot}7\times10^{-4}$M, ●——●, in 14 other experiments. Each point represents the response from a single preparation. The response was determined by comparing the sinus rate achieved at the plateau of the effect with the sinus rate just prior to drug administration. The curves were fitted by eye. Average (±S.E.) initial control sinus rates were 252 (±5; n=28) for ▽---▽, 245 (±3; n=14) for ○---○, and 233 (±4; n=14) for ●——●.

reach the level of statistical significance ($0{\cdot}1>P>0{\cdot}05$; Table 1). Only conduction arrhythmias occurred during anaphylaxis in the presence of burimamide. No extrasystolic activity or ventricular tachycardia were observed. The characteristic decrease in coronary flow rate occurring during anaphylaxis was not significantly modified by the presence of burimamide (Table 2). Neither the amount (Table 3) nor the time-course (Fig. 2A) of histamine release were influenced by burimamide. However, under the same conditions smaller increments in sinus rate were attained in the presence of burimamide. Consequently, the curve describing the relationship between rate of histamine release and increase in sinus rate was shifted to the right by burimamide (Figure 3).

Effects of histamine plus burimamide

The positive chronotropic effect of exogenous histamine or histamine plus burimamide is shown in Figure 4. Burimamide shifted the histamine concentration-effect curve to the right in a concentration-dependent fashion.

Discussion

Our results clearly show that burimamide inhibits the positive chronotropic effect of cardiac anaphylaxis in the guinea-pig. The inhibition is concentration-dependent. This finding is consistent with the facts that the positive chronotropic effect of anaphylaxis is induced by histamine (Levi, 1972) and that in the isolated atria (Black *et al.*, 1972) and in the isolated heart (Fig. 4) of the guinea-pig, burimamide competitively inhibits the positive chronotropic effect of exogenous histamine.

An increase in ventricular automaticity is a constant feature of anaphylaxis in the isolated heart ; this effect was completely prevented by burimamide. A possible explanation of this effect of burimamide is based on observations of the effects of exogenous histamine on cardiac automaticity. Histamine has been shown to initiate automatic activity in the isolated left atrium of the guinea-pig (Penna, Illanes, Ubilla & Mujica, 1959) and to enhance the automatic properties of the sinus node cells of the rabbit heart (Levi & Giotti, 1967) and of Purkinje fibres of the sheep ventricle (Mannaioni, Levi, Ledda & Giotti, 1968). Also burimamide antagonizes the initiation of action potentials by histamine in guinea-pig pace-maker cells depolarized by high extracellular potassium (Levi & Pappano, unpublished observations). Therefore, the absence of multifocal extrasystolic activity and of ventricular tachycardia during anaphylaxis in the presence of burimamide demonstrates the ability of burimamide to antagonize the histamine-induced increase in ventricular automaticity.

The abolition by burimamide of the short-lasting positive inotropic effect of anaphylaxis may again be explained in terms of the antagonism of the effects of immunologically released histamine, since the increase in ventricular contraction during anaphylaxis is very probably histamine-induced (Levi, 1972).

Although burimamide completely prevented the occurrence of automaticity arrhythmias during anaphylaxis, it did not abolish conduction arrhythmias. The impairment of atrioventricular conduction during anaphylaxis is closely correlated with the amounts of histamine released (Levi, 1972) ; also, exogenous histamine prolongs the P–R interval in a dose-dependent fashion (Flacke, Atanackovic, Gillis & Alper, 1967 ; Levi, 1972). Furthermore, the concomitant positive chronotropic

effect of histamine indirectly contributes to its negative dromotropic effect (Levi, 1972). Burimamide effectively antagonizes the positive chronotropic effect of released histamine; consequently, the negative dromotropic effect of histamine was attenuated. In anaphylaxis, therefore, burimamide at a concentration of $2{\cdot}7\times10^{-4}$M delayed the onset and the frequency of conduction arrhythmias. Despite this mild anti-arrhythmic effect, it is evident that burimamide did not counteract the histamine-induced prolongation of the P–R interval or the consequent induction of atrioventricular block.

The decrease in coronary flow rate occurring during anaphylaxis depends not on the amounts of histamine released, but on the duration of the conduction arrhythmias (Levi, 1972). Burimamide did not prevent conduction arrhythmias and only slightly shortened their duration. Therefore, the decrease in coronary flow rate occurring in anaphylaxis in the presence of burimamide can easily be explained in terms of the inability of burimamide to affect conduction arrhythmias and, indirectly, the decrease in coronary flow. The possibility that pharmacological mediators other than histamine may contribute to reduce the rate of coronary flow during anaphylaxis cannot be excluded. If other mediators were indeed involved, then obviously burimamide did not antagonize their action.

From the preceding discussion it emerges that burimamide selectively inhibits the positive chronotropic and inotropic effects of immunologically released histamine, whereas it does not inhibit the negative dromotropic effect. These findings are in agreement with the theory of double histamine receptors formulated by Ash & Schild (1966) and substantiated by Black and co-workers (1972). This theory provides for histamine responses to be mediated by two categories of receptors: H_1, selectively inhibited by the classical antihistamines ('mepyramine-like'), and H_2, selectively inhibited by burimamide (Black *et al.*, 1972). None of the prototypes of each of the five chemical classes of traditional antihistamines (ethanolamines, ethylendiamines, alkylamines, piperazines and phenothiazines) inhibited the positive chronotropic and inotropic effects, whereas all five inhibited the negative dromotropic effect of histamine in the isolated guinea-pig heart (Kuye, 1972; Kuye & Levi, 1972). The hypothesis was therefore advanced that H_1-histamine receptors are located at the atrioventricular node and at the coronary vessels of the guinea-pig heart (Kuye, 1972; Kuye & Levi, 1972). That H_2-histamine receptors may be responsible for the positive chronotropic effect of histamine is strongly suggested by the experiments of Black *et al.* (1972) in the isolated guinea-pig atria and by our findings (Figure 4). Our results show that burimamide selectively inhibits the positive inotropic and chronotropic effects of anaphylaxis, but not the negative dromotropic effect. In view of all these findings, it is very possible that immunologically released histamine acts in the heart at two different receptors: H_1, which is involved in the slowing of atrioventricular conduction, and H_2, which is responsible for the stimulation of sinus rate and ventricular contraction.

In conclusion, our investigation clearly identifies burimamide as an agent without cardiostimulatory properties, which does not interfere with the amount or the time-course of histamine release, but which is capable of selectively inhibiting the cardiostimulatory actions of immunologically released histamine.

We are particularly grateful to Dr. J. W. Black of the Research Institute, Smith, Kline & French Laboratories Ltd., Welwyn Garden City, Herts, for the gift of burimamide. This work was supported by a grant-in-aid of the New York Heart Association and in part by the

U.S. Public Health Service training grant NIGMS GM99. N. C. was a U.S. Public Health Service pre-doctoral trainee in pharmacology.

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(*Received October* 24, 1972)