Br. J. Pharmac. (1976), **57**, 247–249

BLOCKADE BY BURIMAMIDE OF THE RESTORATIVE EFFECT OF HISTAMINE IN TETRODOTOXIN-TREATED HEART PREPARATIONS

F. LEDDA, LAURA MANTELLI & A. MUGELLI
Department of Pharmacology, University of Florence, Italy

1 In isolated heart preparations in which fast sodium channels were blocked by tetrodotoxin ($2-4 \times 10^{-5}$ M), excitability was restored by histamine (6×10^{-6} M to 10^{-5} M).

2 This effect was antagonized by EDTA (2×10^{-6} M), D_{600} compound (0.5 μg/ml) and the H_2-receptor antagonist, burimamide (2×10^{-4} M).

Introduction

The positive inotropic effect of histamine has been largely investigated from the point of view either of the biochemical changes, or of the type of receptor involved in the mechanical response (McNeill & Muschek, 1972; Poch, Kukovetz & Scholz, 1973; McNeill & Verma, 1974; Moroni, Ledda, Fantozzi, Mugelli & Mannaioni, 1974).

On the other hand, few results of electrophysiological studies on the mechanism of the inotropic response of the amine are at present available (Houki, 1973; Watanabe & Besch, 1974). In the present study we attempted to demonstrate that histamine is able to activate the slow inward calcium current in heart preparations in which fast sodium channels were blocked by tetrodotoxin, and that this effect of the amine is mediated by H_2-receptors.

Methods

Ventricular strips of guinea-pig heart were placed in a 15 ml chamber containing Tyrode solution of the following composition (mM): Na^+ 149.3, K^+ 2.7, Ca^{++} 1.8, Mg^{++} 1.05, Cl^- 145.4, HCO_3^- 11.9, $H_2PO_4^-$ 0.4, D glucose 5.6. The solution was aerated with a gas mixture of 97% O_2 and 3% CO_2. The pH of the solution was 7.5 and the temperature kept constant at 32°C. The preparations were paced at 150 stimuli/min, and transmembrane action potentials were recorded by microelectrodes filled with 3 M KCl (resistance about 10 megohms).

The first derivative of the depolarization phase was obtained by a differentiator (operational amplifier) with a linear output between 100 and 1000 V/s. The preparations were first treated with tetrodotoxin ($2-4 \times 10^{-5}$M) and became inexcitable in a few minutes. At this time the stimulation rate was reduced to 30/min and the stimulus strength and duration were doubled. Increasing concentrations of histamine (histamine dihydrochloride, Calbiochem) were then added to the perfusion fluid at 10 min intervals, and the minimal dose able to restore the excitability was checked.

The following drugs were used in order to control the specificity of the observed effect: ethylenediaminetetraacetic acid (EDTA, Merck), D_{600} hydrochloride (kindly supplied by Knoll) and burimamide (kindly supplied by Smith, Kline & French Laboratories).

Results

Histamine at the higher concentration used (10^{-5} M) was unable to restore the excitability of tetrodotoxin-treated preparations when the original stimulus parameters were used. However if the stimulus strength and duration were doubled and the stimulation rate was reduced from 150 to 30/min, electrical activity as well as contraction were restored by high concentrations of histamine (6×10^{-6} M in one experiment and 10^{-5} M in three experiments).

In two experiments it was observed that such a restoration of excitability was blocked by D_{600} (0.5 μg/ml; Figure 1) or by EDTA (2×10^{-6} M).

Moreover burimamide (2×10^{-4} M), when applied after histamine, constantly abolished the restoration of excitability induced by the amine (Figure 2).

Discussion

Demonstration that the restoration of action potentials by histamine in tetrodotoxin-treated preparations is dependent on activation of the slow calcium inward current was obtained in the present study by the

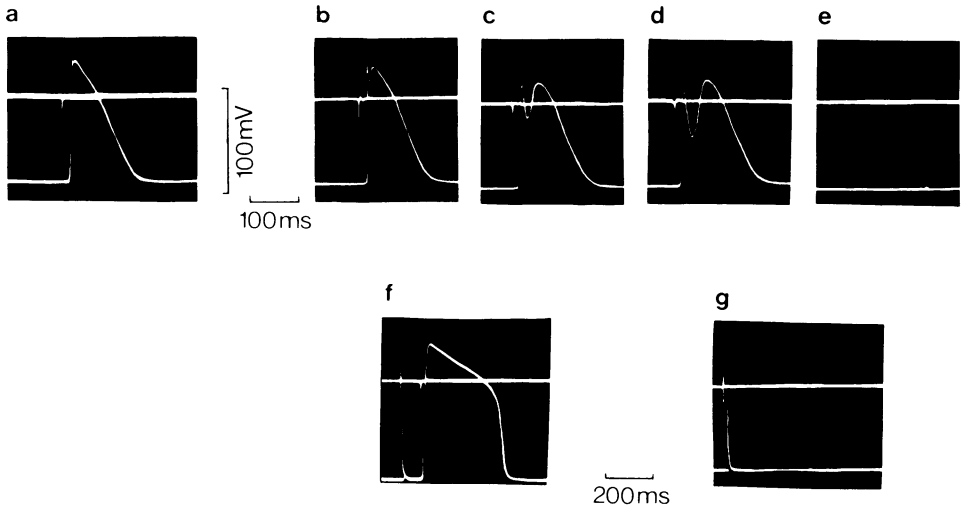

Figure 1 Transmembrane action potentials of strips of guinea-pig ventricular heart muscle. *Upper row*: Stimulation rate 150/min; (a) control; (b), (c), (d) and (e) at 60, 90, 120 and 180 s respectively after treatment with tetrodotoxin (2×10^{-5} M). *Lower row*: Stimulation rate 30/min; (f) 10 min after histamine (10^{-5} M) in the presence of tetrodotoxin; (g) 10 min after D_{600} (0.5 μg/ml) in the presence of histamine and tetrodotoxin as in (f). Incubation temperature, 32 °C.

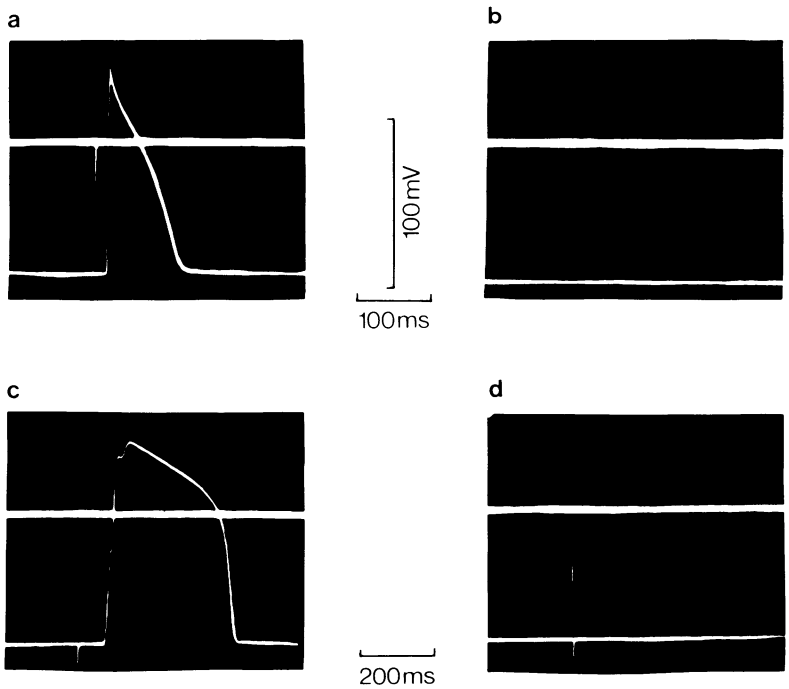

Figure 2 Transmembrane action potentials of strips of guinea-pig ventricular heart muscle. *Upper row*: Stimulation rate 150/min; (a) control; (b) 3 min after treatment with tetrodotoxin 2×10^{-5} M. *Lower row*: Stimulation rate 30/min; (c) 10 min after histamine (10^{-5} M) in the presence of tetrodotoxin; (d) 10 min after burimamide (2×10^{-4} M) in the presence of histamine and tetrodotoxin as in (c). Incubation temperature, 32 °C.

chelation of extracellular calcium with EDTA and by the use of the specific inhibitor of calcium transmembrane influx D_{600} (Kolhardt, Bauer, Krause & Fleckenstein, 1972).

Therefore it is possible to conclude that histamine was able to increase calcium inward current in myocardial fibres, as previously shown by Houki (1973) in potassium-depolarized heart preparations.

Moreover the H_2-receptor blocking drug burimamide (Black, Duncan, Durant, Ganellin & Parsons, 1972) effectively antagonized the increase in calcium influx induced by activation of histamine receptors.

This observation is in good agreement with the well-known antagonism of the effects of histamine on cardiac muscle and cyclic AMP levels by H_2-receptor blocking drugs (Poch *et al.*, 1973; McNeill & Verma, 1974; Moroni *et al.*, 1974).

References

BLACK, J.W., DUNCAN, W.A.M., DURANT, C.J., GANELLIN, C.R. & PARSONS, M.E. (1972). Definition and antagonism of histamine H_2 receptors. *Nature,* **236**, 385–390

HOUKI, S. (1973). Restoration effects of histamine on action potential in potassium-depolarized guinea-pig papillary muscle. *Arch. int. Pharmacodyn.,* **206**, 113–120.

KOLHARDT, M., BAUER, B., KRAUSE, H. & FLECKENSTEIN, A. (1972). Differentiation of the transmembrane Na and Ca channels in mammalian cardiac fibres by the use of specific inhibitors. *Pfluger's Arch.,* **335**, 309–322.

McNEILL, J.H. & MUSCHEK, L.D. (1972). Histamine effects on cardiac contractility, phosphorylase and adenyl cyclase. *J. mol. cell. Cardiol.,* **4**, 611–624.

McNEILL, J. H. & VERMA, S.C. (1974). Blockade by burimamide of the effects of histamine and histamine analogs on cardiac contractility, phosphorylase activation and cyclic adenosine monophosphate. *J. Pharmac. exp. Ther.,* **188**, 180–188.

MORONI, F., LEDDA, F., FANTOZZI, R., MUGELLI, A. & MANNAIONI, P.F. (1974). Effects of histamine and noradrenaline on contractile force of guinea pig ventricular strips: antagonism by burimamide and metiamide. *Agents and actions,* **4**, 314–319.

POCH, G., KUKOVETZ, W. R., & SCHOLZ, N. (1973). Specific inhibition by burimamide of histamine effects on myocardial contraction and cyclic AMP. *Naunyn Schmiedeberg's Arch. Pharmac.,* **280**, 223–228.

WATANABE, A.M. & BESCH, H.R. (1974). Cyclic adenosine monophosphate modulation of slow calcium influx channels in guinea pig heart. *Circ. Res.,* **35**, 316–324.

(Received October 27, 1975, Revised December 12, 1975)