Br. J. Pharmac. (1978), **62**, 321–324

β-ADRENOCEPTOR MEDIATED INHIBITION BY TERBUTALINE OF HISTAMINE EFFECTS ON VASCULAR PERMEABILITY

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1 In guinea-pigs, previously given Evans blue dye intravenously, 13 to 18 intradermal injections of histamine, with or without other drugs, were made into the depilated trunk skin. Dye was then quantitatively extracted from each skin area and the measured absorbance values were used as a measure of vascular leakage of macromolecules.

2 Histamine (0.5 to 12 nmol) produced dose-related increases in vascular leakage. These were reduced by terbutaline (1 and 10 nmol) which produced a significant shift in the histamine dose-response lines to lower absorbance values. The effect of 0.1 nmol of terbutaline was significant only against doses of histamine of less than 2 nmol.

3 Propranolol (1 nmol and 10 nmol) antagonized the effects of terbutaline. Propranolol, at a dose of 10 nmol but not 1 nmol, itself reduced the responses to 1.5 nmol histamine.

4 We conclude that the inhibition by terbutaline of histamine-induced dye leakage in guinea-pig skin is mediated by stimulation of β-adrenoceptors and it is suggested that this effect of terbutaline occurs directly on β-receptors at the vascular leakage site.

Introduction

There is some evidence that β-adrenoceptor stimulants inhibit the vascular leakage of large molecules produced by various mediators. Green (1972) showed that isoprenaline, adrenaline, noradrenaline and salbutamol antagonized the leakage of protein-bound Evans blue dye into the mouse peritoneal cavity following intraperitoneal injections of bradykinin, histamine or 5-hydroxytryptamine. Isoprenaline was more potent than noradrenaline and the effects were antagonized by β-adrenoceptor blocking drugs but not by α-adrenoceptor blocking drugs. Therefore, the inhibition of leakage by the β-adrenoceptor stimulants may be due to an action on β-adrenoceptors. Svensjo, Persson & Arfors (1976), using a hamster cheek pouch preparation, demonstrated a reduction by terbutaline and isoprenaline of leakage of fluorescein isothiocyanate dextran produced by bradykinin. Although they did not examine the effect of β-adrenoceptor blocking drugs, these workers showed that terbutaline did not diminish local blood flow in the preparation, suggesting that leakage was inhibited by a direct effect of terbutaline on the venular endothelial cells. A similar suggestion was put forward by Green (1972).

In view of the contribution which the inhibition of leakage may make in the overall action of β-adrenoceptor stimulants, the present study was undertaken to evaluate the effects of terbutaline on dye leakage induced by histamine in the skin of guinea-pigs injected with Evans blue dye. The effect of a β-adrenoceptor blocking drug on the inhibition by terbutaline was also investigated.

A preliminary account of this work was presented to the annual meeting of the Australasian Society of Clinical and Experimental Pharmacologists in November 1976 (Persson & O'Donnell, 1977).

Methods

The trunk skin of conscious guinea-pigs (360–729 g) was shaved and depilated with a paste described by Miles & Miles (1952). The depilated skin areas were washed thoroughly with warm water and each animal was then dried and left overnight in a constant temperature environment (22 to 24°C). On the next day, each animal was injected with 30 mg/kg of Evans blue dye into a marginal ear vein. Thirty min later, 13 to 18 randomized constant volume (0.1 ml) injections of 0.9% w/v NaCl solution (saline), histamine, or histamine with other drugs, were injected intradermally into the trunk skin of each animal. The thin skin 30–40 mm on each side of the ventral mid-line was

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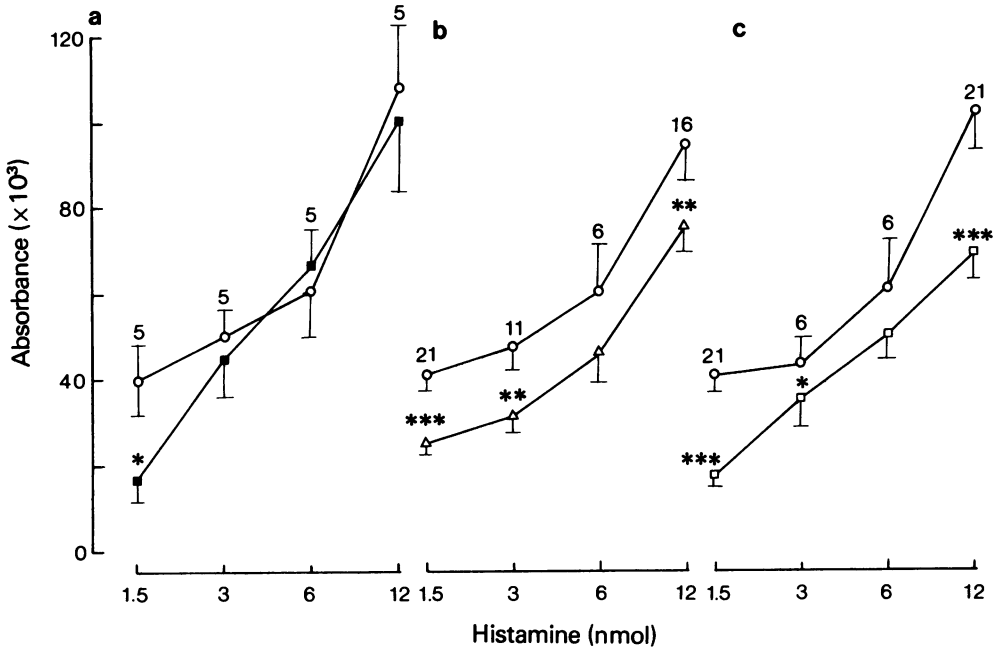

Figure 1 Reduction by terbutaline of dye leakage caused by histamine in guinea-pig skin. Mean optical absorbances and s.e. mean are given for histamine alone (○) and in (a) in the presence of terbutaline 0.1 nmol (■); in (b) terbutaline 1 nmol (△) and in (c) terbutaline 10 nmol (□). The number of animals is indicated above the histamine control values. Asterisks indicate that terbutaline significantly reduced histamine responses (paired t test; * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$).

avoided (Miles & Miles, 1952). Thirty min after the last intradermal injection, the animals were killed by a blow on the head, bled, and the skin containing the injected areas removed. Dye was extracted from blue areas as described by Harada, Takeuchi, Fukao & Katagiri (1971). For this, each blue area of skin was cut into approximately 10 pieces; these were placed in a mixture of 3 ml 0.5% aqueous sodium sulphate and 7 ml acetone in a sealed tube, and then incubated at 25°C for 24 h with occasional shaking. After centrifugation, the absorbance of the supernatant liquid was measured at 620 nm.

Each animal received a saline injection, doses of histamine (controls) and doses of histamine with terbutaline, so that the effects of terbutaline on histamine responses could be assessed on the same animal. The same principle was followed in experiments with propranolol: doses of histamine plus propranolol (controls for propranolol) and of histamine with propranolol plus terbutaline were administered to each animal. In each animal, the absorbance value obtained from the saline-injected area was deducted from other absorbance values. Solutions of histamine acid phosphate (BDH), propranolol hydrochloride (ICI) and terbutaline sulphate (Astra) were made up fresh each day in saline and kept at 4°C during the experiment.

Mean absorbance values were plotted against the log dose of histamine. Pairs of dose-response lines to histamine were compared by a 2-way analysis of variance (Kirk, 1968). This provided F-values from which it could be established whether a) there was a significant regression of the histamine dose-response line and b) terbutaline produced a significant shift in the histamine dose-response line, either in the absence or in the presence of propranolol. A paired t test was used to obtain significance levels for differences between paired absorbance readings at individual dose levels of histamine. The Figures show mean values ± s.e.mean.

Results

Histamine (0.5 to 12 nmol) injected intradermally into guinea-pig skin produced a dose-related leakage of Evans blue dye (Figures 1 and 2). Although all injections caused a small area of traumatic blueing at the centre of the injection area, the round areas of blueing produced by histamine were clearly distinguishable

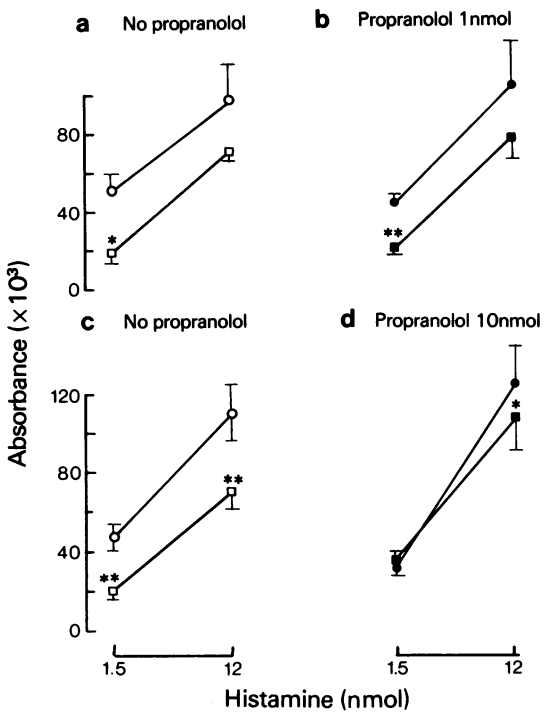

Figure 2 Effects of propranolol on reduction of leakage by terbutaline in guinea-pig skin. The left hand graphs (a and c) show absorbance values produced by histamine (○) and their reduction by 10 nmol of terbutaline (□). The right hand graphs (b and d) show the effects of histamine (●) and of histamine plus terbutaline (■) obtained in the same animals but in the presence of 1 nmol of propranolol (b, $n = 5$) and of 10 nmol of propranolol (d, $n = 10$). Asterisks (paired t test) indicate that terbutaline significantly reduced histamine responses (* $P < 0.05$, ** $P < 0.01$). The figure illustrates that both doses of propranolol antagonized the effects of 10 nmol of terbutaline in that the significant shift in the histamine dose-response line seen in the absence of propranolol ($P < 0.01$ in a; $P < 0.001$ in c) was *not* significant in the presence of propranolol.

from saline injections. There was a significant regression in the histamine dose-response line over the dose range 1.5 to 12 nmol histamine ($P < 0.001$). Doses of histamine greater than 12 nmol were not used because considerable subcutaneous leakage occurred.

Terbutaline (1.0 and 10 nmol but not 0.1 nmol) caused a significant shift in the histamine dose-response line to lower absorbance values (Figure 1) i.e. reduced leakage (1.0 nmol, $P < 0.001$; 10 nmol, $P < 0.01$). There was no significant difference between the effects of 1.0 and 10 nmol of terbutaline. Because 0.1 nmol of terbutaline significantly reduced the leakage to 1.5 nmol of histamine in these experiments ($t = 3.62$, $P < 0.05$) this dose of terbutaline was re-examined in a separate series of experiments in which only low doses of histamine were used (0.5, 1 and 2 nmol). Terbutaline (0.1 nmol) caused a shift in this histamine dose-response line although it was not statistically significant.

Propranolol (1 nmol and 10 nmol) antagonized the effects of 10 nmol (Figure 2) and 1.0 nmol of terbutaline. Propranolol at a dose of 10 nmol but not of 1 nmol, itself significantly reduced responses to 1.5 nmol of histamine ($t = 3.15$, $P < 0.05$). Histamine responses in the presence of terbutaline and 10 nmol propranolol were significantly larger than histamine responses in the presence of terbutaline alone (1.5 nmol of histamine, $t = 4.28$, $P < 0.01$; 12 nmol of histamine, $t = 3.39$, $P < 0.01$).

In all experiments (Figures 1 and 2) the F value for the interaction term (histamine doses × terbutaline doses) was not statistically significant, supporting the observation that terbutaline produced a parallel shift in the histamine dose-response line.

Discussion

Majno, Gilmore & Leventhal (1967) showed, in vessels of exposed striated muscle, how histamine and other mediators increased microvascular permeability to large molecules by contracting venular endothelial cells as a result of which gaps formed between cells. Electron-microscopic studies have indicated that this mechanism of leakage can operate in other tissues including the hamster cheek pouch (Hultström & Svensjö, 1977) and dog lung (Pietra, Szidon, Leventhal & Fishman, 1971). The possibility that β-adrenoceptor stimulants might relax endothelial cells, oppose gap formation and thus decrease vascular permeability was first considered by Green (1972) and substantiated by Svensjö *et al.* (1976). This concept is compatible with the general property of β-adrenoceptor stimulants of decreasing tension in contractile tissues e.g. various types of smooth muscle (Axelsson, 1971), mammalian heart muscle (Morad & Rolett, 1972) and slow contracting skeletal muscle (Bowman & Zaimis, 1958). It may explain the present findings that terbutaline, a β-adrenoceptor stimulant, inhibited the increase in permeability produced by histamine in guinea-pig skin. The view that terbutaline might cause this inhibition by a direct action on β-adrenoceptors in the venular endothelium was supported by the finding that the effects of terbutaline were antagonized by propranolol. The higher dose of propranolol (10 nmol) itself reduced, like terbutaline, the responses to histamine and it could be argued that the

propranolol was obscuring rather than antagonizing the effects of terbutaline. This argument could be discounted by the observation that histamine responses in the presence of terbutaline and 10 nmol propranolol were *larger* than histamine responses in the presence of terbutaline alone. Furthermore, significant antagonism of terbutaline was also seen with a lower dose of propranolol (1 nmol) which itself had no significant effect on histamine responses. The mechanism of this reducing effect of 10 nmol propranolol is unknown.

If terbutaline and other β-adrenoceptor stimulants can oppose mediator-induced leakage by a direct action on β-receptors in the venular endothelium, this may affect the interpretation of experiments in which the anti-anaphylactic effects of β-adrenoceptor stimulants are examined utilizing skin dye leakage (e.g. Mielens, Ferguson & Rosenberg, 1974). The inhibitory mechanism of the β-adrenoceptor stimulants may involve not only the β-receptors concerned in the release of the endogenous mediators, but also antagonism of the effects of these mediators directly at the site of venular leakage. In addition, if the β-adrenoceptor stimulants are able, in general, to antagonize endothelial contraction produced by mediators, this may contribute to their action in asthma where anaphylactic reactions in the lung produce a number of chemical mediators, many of which increase microvascular permeability. Suppression of the mucosal and submucosal oedema which occurs in the asthmatic lung (Dunnill, 1975) would be a useful addition to the other effects of these drugs in asthma viz. bronchodilatation, increase in the rate of mucociliary clearance and inhibition of the release of mediators.

This work was supported by a grant from the National Health and Medical Research Council of Australia and this aid is gratefully acknowledged.

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(Received March 7, 1977.
Revised September 8, 1977.