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TIME COURSES OF THE ANTI-ANAPHYLACTIC AND ANTI-INFLAMMATORY EFFECTS OF DEXAMETHASONE IN THE RAT AND MOUSE

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1 The time course of the activity of dexamethasone has been studied in a variety of anaphylactic and inflammatory reactions in the rat and the mouse.

2 The times of peak activity of dexamethasone, expressed as time between oral dosage and induction of response, and the approximate ED_{50} values (mg/kg) found were: anaphylactic bronchoconstriction in rats, 12–24 h, ED_{50} 1.8; passive cutaneous anaphylaxis in rats, 6 h, ED_{50} 0.04; cutaneous reactions to histamine in rats, 4 h, ED_{50} 0.01; carrageenin-induced paw oedema in rats, 4 h, ED_{50} 0.03; pinnal anaphylaxis in mice, 6 h, ED_{50} 0.82; histamine-induced pinnal reactions in mice, 6 h, ED_{50} 0.05.

3 In rats, the characteristics of the inhibitory effects of dexamethasone indicate a differential activity against anaphylactic and inflammatory reactions and between different types of inflammatory reactions. Possible reasons for these differences are discussed.

4 In mice there was little difference between the inhibition by dexamethasone of cutaneous allergic and histamine-induced reactions.

5 Pinnal anaphylaxis in mice was potentiated by dexamethasone given 1–2 h before challenge.

Introduction

Clinical experience in the treatment of bronchial asthma by glucocorticoids has indicated that there is a time lag between administration of the drug and onset of improvement. Reports of the length of this time lag have varied from 2 to 24 h (Schwartz, 1951; Herxheimer, 1966; Cope, 1972; Ellul-Micallef, Borthwick & McHardy, 1974; Ellul-Micallef & French, 1975).

A similar time lag is observed in experimental anaphylaxis although the time course has not been studied. In rats, anaphylactic mortality is reduced more effectively when dexamethasone is given 18 h rather than 2 h before challenge (Fregnan & Suchowsky, 1968) and rat anaphylactic bronchoconstriction is suppressed most effectively when dexamethasone is given 24 h before antigen (Church, Collier & James, 1972). In mice, anaphylactic mortality is reduced when dexamethasone is given 18 to 24 h before challenge but not when given 1 to 2 h beforehand (Fregnan & Suchowsky, 1968; Laddu & Sanyal, 1968; Dietrich, Komarek & Pericin, 1971).

In contrast, hydrocortisone is very effective in relieving non-anaphylactic local oedema when given 1 h before injection of a number of local irritants (Garattini, Jori, Bernardi, Carrara, Paglialunga & Segre, 1965).

In this paper we describe the time course with which dexamethasone inhibits antigen-induced anaphylactic reactions and inflammatory reactions induced by histamine and carrageenin in the rat and mouse.

Methods

Animals and materials

Male wistar rats (CFHB, Carworth) or male mice (CFLP, Carworth) were used.

The following substances, calculated as base, were dissolved or suspended in 0.9% w/v sodium chloride solution (saline) or water: aluminium hydroxide gel

(prepared by mixing equal volumes of 2 N $Al_2(SO_4)_3$ and 2 N NaOH and washing the collected precipitate three times with saline). *Bordetella pertussis* vaccine B.P. (Wellcome), carrageenin (Fisons), dexamethasone (Roussel), Evans blue dye (Fluke), histamine acid phosphate (Koch Light), horse serum (No. 3, Wellcome) and ovalbumen (BDH) were also used.

Experiments in rats

Anaphylatic bronchoconstriction. Rats weighing 150 to 175 g were sensitized by intraperitoneal injection of ovalbumen 100 μg, in aluminium hydroxide gel, 1 milligram. Fourteen days later they were challenged intravenously with ovalbumen, 0.5 to 2.0 milligrams. Tracheal flow was recorded for 10 min and percentage bronchoconstriction estimated as described by Church *et al.*, (1972).

Passive cutaneous anaphylaxis (PCA). Antiserum to ovalbumen was raised in donor rats by the method of Orr & Blair (1969). Rats weighing 150 to 175 g were sensitized with ovalbumen, 1 mg intramuscularly, and *B. pertussis*, 2×10^{10} organisms (adjuvant) intraperitoneally. Ten days later rats were infected with *Nippostrongylus brasiliensis*, 4000 larvae subcutaneously, to increase IgE production (Orr & Blair, 1969) and the serum collected after a further 14 days. This serum had a PCA titre of 120–160.

Recipient rats, weighing 180 to 200 g, received intradermal injections of 0.1 ml of a 1/25 dilution of antiserum into four discrete areas on the shaved back. They were challeged 72 h later by intravenous injection of 1 ml of a solution containing ovalbumen 1 mg, and Evans blue dye, 5 milligrams. The resultant blue spots were scored 30 min later on an arbitrary 0 to 4 scale by examination of the underside of the skin by a 'blind' observer.

Histamine-induced cutaneous reactions. Intradermal injections of 0.05 ml of solutions of histamine, 100 μg/ml, were made into four discrete areas of the shaved back of rats weighing 180 to 200 grams. Evans blue dye, 0.5 ml of a 1% solution, was injected intravenously immediately before histamine. The resultant blue spots were scored as in the PCA tests. The blueing reaction to intradermal saline, 0.05 ml, included as a control in each animal, was subtracted from the mean reaction obtained after histamine.

Paw oedema. Inflammation and oedema were induced in the right hind paw of rats weighing 100 to 120 g by subplantar injection of 0.1 ml of a 1% suspension of carrageenin. The fractional oedema volume measured by plethysmography (Ugo Basile, Milan) 3 h after carrageenin injection was calculated by the formula:

$$\frac{\text{Paw volume at 3 h} - \text{initial paw volume}}{\text{Initial paw volume}}$$

Experiments in mice

Pinnal anaphylaxis. Mice weighing 16 to 20 g were sensitized to horse serum, challenged and pinnal anaphylaxis measured as described by Church, James & Miller (1974).

Histamine-induced pinnal reactions. Pinnal reactions to histamine were induced in mice weighing 16 to 20 g by stabbing the pinnae through a solution of histamine, 600 μg/ml, as previously described (Church & Miller, 1975).

Dosing schedules and group sizes

In time course experiments, corticosteroid was given orally 1, 2, 4, 6, 12, 24 or 48 h before challenge to groups of 8 to 10 rats (anaphylactic bronchoconstriction), 7 rats (PCA, histamine-induced cutaneous inflammation and paw oedema) or 9 mice (pinnal anaphylaxis and histamine-induced pinnal inflammation). In all experiments, three similar sized control groups were given water orally 2, 12 or 24 h before challenge. As these groups were never statistically different the drug effects were expressed as a percentage inhibition of the pooled control.

Calculation of standard error of percentage inhibition

The approximate standard error of each percentage inhibition was calculated by the following equation derived from Feiller's theorem (Colquhoun, 1971):

$$\text{s.e. of \% inhibition} = 100\ m\ \sqrt{(Cx)^2 + (Cy)^2}$$

where Cx is the coefficient of variation of the control mean response, Cy is the coefficient of variation of the test mean response and m is

$$\frac{\text{mean test response}}{\text{mean control response}}$$

Results

Experiments in rats

Anaphylactic bronchoconstriction. In two experiments, dexamethasone, 5 mg/kg, produced a highly significant ($P < 0.001$) inhibition of anaphylactic bronchoconstriction when given 12 or 24 h before challenge (Figure 1). Results obtained at other times after dosage were not significant ($P > 0.05$). The greatest inhibition, 51.3 ± 13.5%, was observed at 24 h

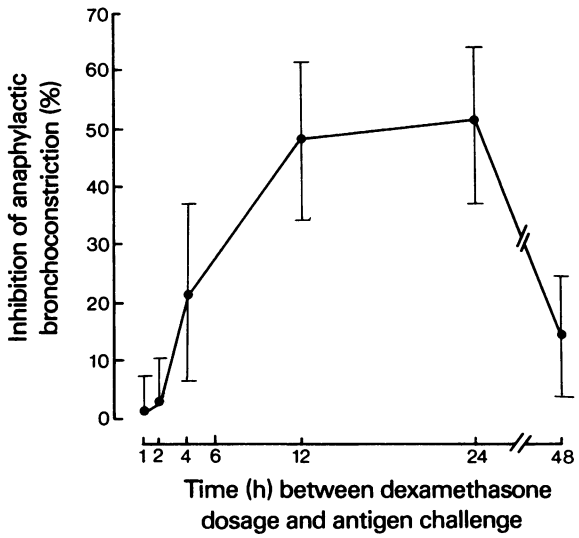

Figure 1 Inhibition of anaphylactic bronchoconstriction in the rat by dexamethasone given at various times. Dexamethasone, 5 mg/kg, was given orally to groups of 8 to 10 sensitized rats at various times before intravenous challenge with ovalbumen and recording of bronchoconstriction. The vertical bars indicate s.e. mean. Control animals showed 83.6 ± 3.5% bronchoconstriction.

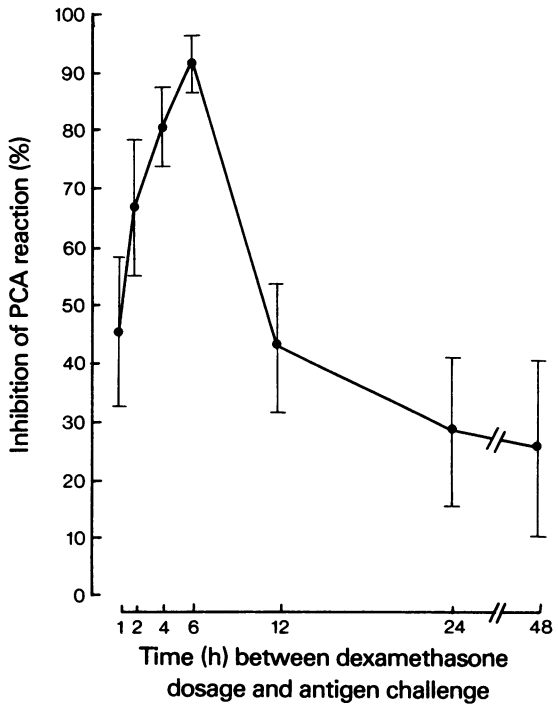

Figure 2 Inhibition of passive cutaneous anaphylaxis (PCA) in the rat by dexamethasone given at various times. Dexamethasone, 0.1 mg/kg, was given orally to groups of 7 passively sensitized rats at various times before intravenous challenge with ovalbumen, 1 mg. Passive cutaneous reactions were measured 30 min after challenge. The vertical bars indicate s.e. mean. Control animals gave a score of 2.51 ± 0.18 on the 0 to 4 scale.

after dosage when the ED_{50} (the dose calculated to inhibit the response by 50%) was 1.8 mg/kg.

Passive cutaneous anaphylaxis. In two experiments, dexamethasone, 0.1 mg/kg, produced a highly significant ($P < 0.001$) inhibition of passive cutaneous anaphylaxis when given 1, 2, 4, 6 or 12 h before challenge (Figure 2). When given 24 or 48 h before challenge dexamethasone inhibited less effectively, though still significantly ($P < 0.05$). The maximum inhibition by dexamethasone, 91.7 ± 5.4%, was observed 6 h after dosage when the ED_{50} was 0.04 mg/kg.

Histamine-induced cutaneous reactions. Dexamethasone, 0.1 mg/kg produced a highly significant $P < 0.001$) inhibition of skin blueing when given 2, 4, 6 or 12 h before intradermal injection of histamine, 5 μg (Figure 3). When given 24 h before histamine dexamethasone had a weaker effect ($P < 0.05$) and 1 or 48 h before histamine had no significant activity. The maximum inhibition by dexamethasone, 73.7% ± 5.6, was observed 4 h after dosage when the ED_{50} was approximately 0.01 mg/kg.

Carrageenin-induced paw oedema. Dexamethasone, 1.0 mg/kg, produced a highly significant ($P < 0.001$) inhibition of paw oedema when given 1, 2, 4 or 6 h before carrageenin (Figure 4). When given 12, 24 or 48 h previously a less significant ($P < 0.05$) inhibition or response was observed. The maximum inhibition, 84.2% ± 7.2 and 82.5% ± 5.5, was seen when dexamethasone was given 1 or 2 h before carrageenin, when the ED_{50} was 0.03 mg/kg. Because of the slow development of the reaction (3 h) the true lag time to peak activity could not be accurately determined but only assumed to be less than 4 hours.

Experiments in mice

Pinnal anaphylaxis. When given at a dose of 5 mg/kg 4, 6, 12 or 24 h before challenge in two experiments, dexamethasone produced a highly significant ($P < 0.001$) inhibition of pinnal anaphylaxis, the maximum inhibition occurring at 6 h (71.7% ± 4.1) and 12 h (73.9% ± 2.8) (Figure 5). The ED_{50} at 6 h was 0.82 mg/kg. The inhibition was less significant ($P < 0.05$) at 48 h and not significant at 1 and 2

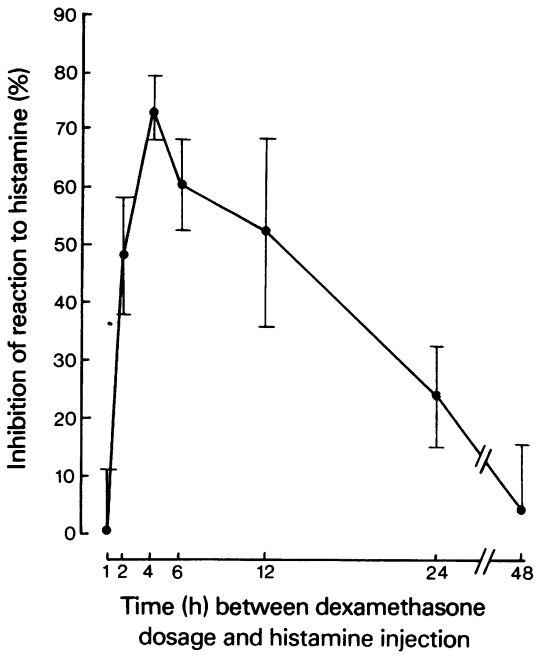

Figure 3 Inhibition of histamine-induced inflammation in rat skin by dexamethasone given at various times. Dexamethasone, 0.1 mg/kg, was given orally to groups of 7 rats at various times before intradermal injection of histamine, 5 μg. The reactions were allowed to develop for 30 min before measurement. The vertical bars indicate s.e. mean. Control animals gave a score of 2.14 ± 0.11 on the 0 to 4 scale.

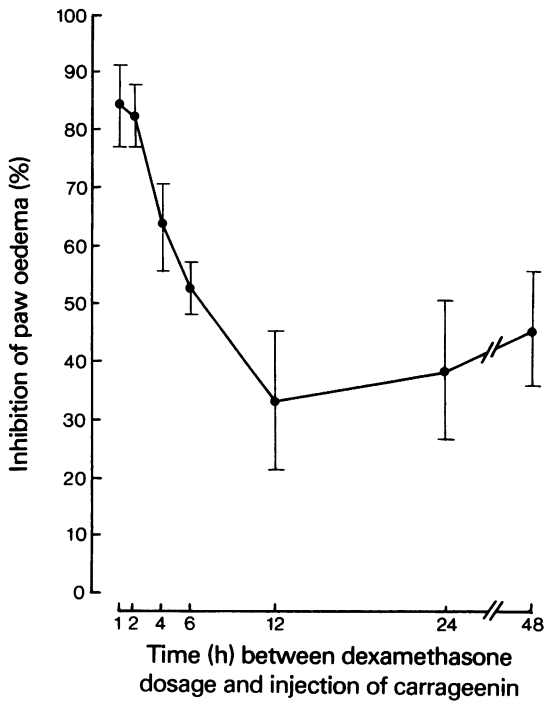

Figure 4 Inhibition of paw oedema in the rat by dexamethasone given at various times. Dexamethasone, 1 mg/kg, was given orally to groups of 7 rats at various times before subplantar injection of carrageenin. The reactions were allowed to develop for 3 h before measurement. The vertical bars indicate s.e. mean. Control animals gave a fractional oedema volume of 0.57 ± 0.05 units.

hours. Dexamethasone, 0.1 mg/kg, produced maximum inhibition, 41% ± 11.5, at 6 hours.

When the mice were challenged 1 to 2 h after dexamethasone, pinnal anaphylaxis was potentiated. After 5 mg/kg of dexamethasone, the response was slightly ($P > 0.05$) enhanced at 1 h, and after 0.1 mg/kg it was potentiated by 33.9% ± 16.1 ($P < 0.05$) at 2 hours.

Histamine-induced pinnal reactions. Dexamethasone, 5 mg/kg, produced a highly significant ($P < 0.001$) inhibition of pinnal inflammation when given 4, 6, 12 or 24 h before intradermal injection of histamine (Figure 6). The inhibition was less significant ($P < 0.05$) at 2 or 24 h and not significant at 1 hour. Dexamethasone had maximum effects when given 6 h (78.8% ± 3.6) or 12 h (79.5% ± 3.8) before histamine. The ED_{50} of dexamethasone at 6 h was approximately 0.5 mg/kg. Dexamethasone, 0.1 mg/kg, produced maximum inhibition, 42.6% ± 8.4, at 4 h and did not potentiate the response at 1 to 2 hours.

Discussion

The results obtained in these experiments show that the duration of the lag period betweeen the administration of dexamethasone and its suppression of anaphylactic and inflammatory reactions differ in the various tests used. However, an accurate comparison of doses is not valid as the severity of the reactions is not necessarily comparable. The suppression by corticosteroid of these reactions is probably the sum of many activities, each of which may have a different time course.

Two basic effects of dexamethasone may be considered: inhibition of mediator release and modification of the response of the target cells to mediators. Rat anaphylactic bronchoconstriction, which is due to the release and action of 5-hydroxytryptamine and possibly slow reacting substance of anaphylaxis (SRS-A) (Church *et al.*, 1972; Farmer, Richards, Sheard & Woods, 1975) is inhibited only by a high

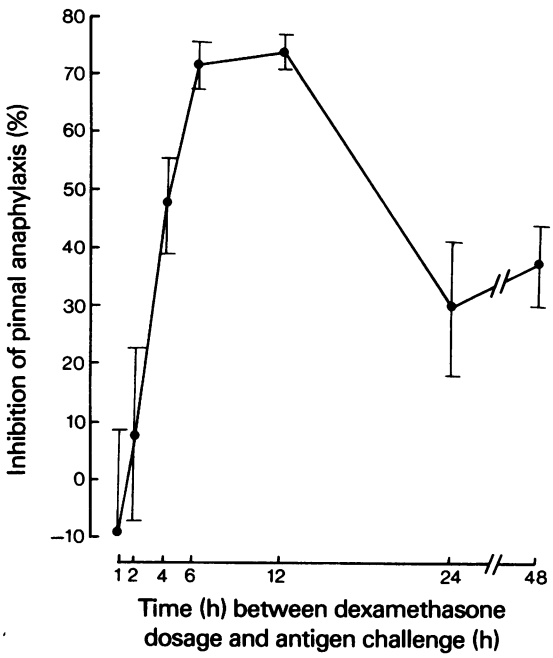

Figure 5 Inhibition of pinnal anaphylaxis in the mouse by dexamethasone given at various times. Dexamathasone, 5 mg/kg, was given orally to groups of 9 sensitized mice at various times before stabbing the pinnae through a drop of neat horse serum. The reactions were allowed to develop for 30 min before measurement. The vertical bars indicate s.e. mean. Control animals gave a mean area of blueing of 32.8 ± 2.6 mm^2.

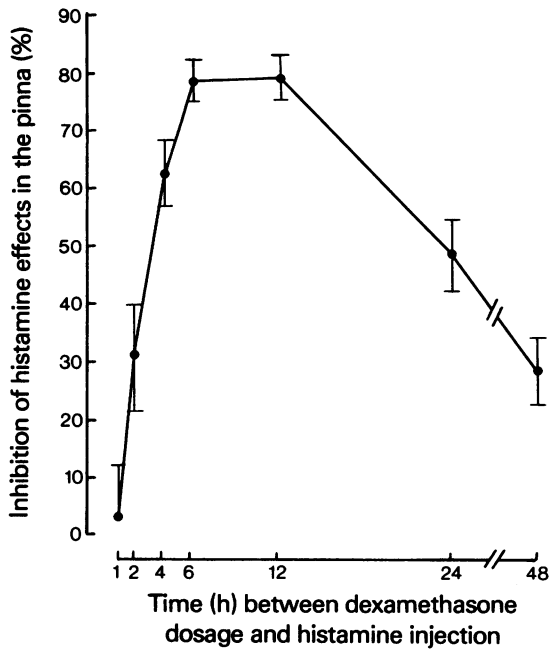

Figure 6 Inhibition of histamine-induced reactions in the mouse pinna by dexamethasone given at various times. Dexamethasone, 5 mg/kg, was given orally to groups of 9 mice at various times before stabbing the pinnae through drops of histamine, 600 μg/ml. The reactions were allowed to develop for 30 min before measurement. The vertical bars indicate s.e. mean. Control animals gave a mean area of blueing of 27.9 ± 1.6 mm^2.

dose of dexamethasone and after a long lag period, possibly because the steroid depresses the release of mediators. Dexamethasone does not reduce the bronchoconstrictor effects of exogenous 5-hydroxytryptamine or SRS-A (Church, 1975).

Intradermal injection of histamine into rat skin, against which dexamethasone was maximally active at 4 h and required only a small dose, does not involve release of mediators but assesses the effect of dexamethasone on the target cells, in this case the capillary bed where corticosteroid may decrease capillary permeability (Benditt, Schiller, Wong & Dorfman, 1950) or cause vasoconstriction (Barry & Woodford, 1974).

Passive cutaneous anaphylaxis in the rat is initiated by the anaphylactic release of mediators from cutaneous mast cells which induce an inflammatory reaction similar to that observed after an injection of histamine, both of which may be inhibited by dexamethasone. That the time course and inhibitory dose correspond more closely to that observed in histamine-induced reactions, suggests that the major effect of dexamethasone in this model is on target organs.

Oedema of the rat paw, measured 3 h after the injection of carrageenin, is thought to result mainly from increased vascular permeability due to the interaction of bradykinin and prostaglandins released during this period (Di Rosa, Papadimitriou & Willoughby, 1971; Ferreira, Moncada, Parsons & Vane, 1974). The short lag period of dexamethasone in this model, less than 4 h, may reflect an action on the capillary bed or an inhibition of prostaglandin release, which has a very short lag period (Gryglewski, Panczenko. Korbut, Grodzinska & Ocetkiewicz, 1975; Flower, Harvey, Moncada, Nijkamp & Vane, 1976; Chang, Lewis & Piper, 1977).

In the mouse pinna, dexamethasone inhibited reactions induced by antigen and histamine with the same time course and the same dose. Thus, no evidence for a differential inhibitory activity of corticosteroid against anaphylactic and histamine-induced reactions in the mouse was detected. However, dexamethasone

given 1 to 2 h before challenge, especially in low doses, potentiated anaphylaxis but not the histamine-induced reaction. This potentiation of pinnal anaphylaxis may be explained by the finding that antigen-induced histamine release from sensitized chopped pinnae *in vitro* is markedly increased when the donor mice are dosed with dexamethasone (0.1 mg/kg) 2 h before removal and challenge of the tissue (unpublished results).

The time course and optimal doses of dexamethasone suggest that its action is multifactorial and depends on the sensitivity to inhibition of the release of the mediators responsible for the reaction and of the target cells in which the reaction is observed.

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