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European Journal of Pharmacology 430 (2001) 305–310

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Absence of mast cell involvement in active systemic anaphylaxis in rats

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Received 19 July 2001; received in revised form 5 September 2001; accepted 13 September 2001

Abstract

This study investigates the role of mast cells in the hypotension induced by antigen-mediated anaphylaxis, compound 48/80 and dextran in mast cell-deficient white spotting (Ws/Ws) and normal wild type (+/+) rats. Rats were sensitized with 10 μg of intraperitoneal ovalbumin in saline or saline alone (sham-sensitized). Sensitized rats, both Ws/Ws and +/+ but not sham-sensitized rats, challenged intravenously with ovalbumin exhibited hypotensive responses. There was no evidence of mast cell activation in rat mesentery 20 min after intravenous antigen challenge in sensitized +/+ rats. Hypotension induced by intravenous injection of dextran (Dextran-162, 6%, 2 ml kg^{-1}) or compound 48/80 (1 mg kg^{-1}) occurred in +/+ rats, but not in Ws/Ws rats, and was inhibited by pretreatment with a combination of chlorpheniramine and cimetidine. Taken together, these data indicate that the hypotensive response induced by antigen-mediated anaphylaxis is independent of mast cell activation, whereas mast cell amines play the main role in the hypotensive response induced by dextran or compound 48/80.

Keywords: Anaphylaxis; Histamine; Mast cell-deficient (Ws/Ws), rat

1. Introduction

Mast cells are regarded as critical effector cells in inflammatory reactions and physiological processes (Galli, 1987; Mekori and Zeidan, 1990; Metcalfe et al., 1997). A variety of mediators derived from mast cells is released in response to immune stimulation and IgE receptor (FcεRI) aggregation as well as through stimulation by biochemical and other signals (Guo et al., 1997; Huang et al., 1998). Convincing evidence for the importance of mast cells in specific immunologic or pathological responses in vivo has been difficult to obtain since other cell types produce many mast cell-associated mediators. It is therefore difficult to predict the net effect of mast cell activation. An optimal model for evaluation of the biological function of mast cells is a system in which one anatomic site contains mast cells and a second similar site does not, thereby making it possible to identify differences in the expression of biological responses between these two sites. Mast cell-deficient rats "white spotting"; Ws/Ws, recently discovered by Niwa et al. (1991), together with their wild type (+/+) littermates as controls, provide a useful model to study the role of mast cells.

Until recently, degranulation of sensitized tissue mast cells was believed to be involved in both local and systemic anaphylactic reactions. But since systemic anaphylaxis can be induced in genetically mast cell-deficient mice (Jacoby et al., 1984; Ha and Reed, 1987), the necessary participation of mast cells in this type of anaphylaxis is now being seriously questioned. The present study was undertaken to determine whether active systemic anaphylaxis can be induced in mast cell-deficient rats and to investigate the role of mediators in the cardiovascular effects caused by antigen, dextran and compound 48/80.

2. Materials and methods

2.1. Preparation and protocols

2.1.1. Animals

Ws/+ heterozygous rats were obtained from the original colony developed by Kitamura. A spontaneous mutation (Ws/+) was identified by Kitamura in a BN/fMai rat colony, and the heterozygous rats were bred with female rats of the Donryu strain to obtain viable Ws/Ws rats (Niwa et al., 1991). As mentioned above, Ws/Ws rats have a 12-base deletion in the tyrosine kinase domain of the *c-kit* gene (Tsujimura et al., 1991) that results in a lack of mast cells. The genotypes were identified by their fur color, which in the homozygous mutants changes to white with black eyes, denoted as white spot pattern (Ws). The non-mutated animals are black, denoted as +/+.

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0014-2999/01/$ - see front matter
PII: S0014-2999(01)01400-5

The animal experiments performed in this study were approved by the regional ethical committee for animal experimentation. The animals were caged separately from other animals in a quiet environment and cages were individually ventilated with filtered air to minimize risk of infection. To obtain offsprings for experiments, female Ws/+ rats (grey fur) were mated with male Ws/Ws rats and Ws/Ws or +/+ rats in the offspring were used at a weight of 230–250 g. All experiments were performed in general anesthesia (see below). At the end of each experiment, animals were killed by an overdose of sodium pentobarbital and exsanguination.

2.1.2. Active sensitization

Rats were sensitized by a single intraperitoneal injection of 10 μg of ovalbumin and 10 mg of aluminum hydroxide (Alhydrogel, Superfos Biosector, Frederikssund, Denmark) as adjuvant in saline or adjuvant and saline alone (sham sensitization). Thirteen days after sensitization, animals were challenged by intravenous injection of ovalbumin (1 mg ml^{-1} at 1 mg kg^{-1}) in saline.

2.1.3. Cardiovascular measurements

Male Wistar, Ws/Ws and +/+ rats, weighing 230–250 g, were first anaesthetized with sodium pentobarbital (60 mg kg^{-1} i.p.). Tracheal cannulation was made to facilitate spontaneous breathing, and subsequently additional pentobarbital (30 mg kg^{-1} i.p.) was given. The carotid artery and jugular vein were cannulated, and catheters inserted were used for systemic blood pressure recording and drug injections, respectively. The systolic (sBP) and diastolic (dBP) blood pressures and heart rate were measured by a pressure transducer connected to a polygraph (Grass Instrument, Quincy, MA, USA). Mean arterial pressure (MAP) was calculated as MAP = dBP + (sBP − dBP)/3. Body temperature was maintained at 37 °C by a heating pad connected to a rectal thermistor.

2.1.4. Experimental protocol

Animals were allowed a 20-min stabilization period whereafter registration of cardiovascular data commenced. Thirty minutes thereafter (time 0) ovalbumin, dextran or compound 48/80 was administered. All other drugs were administered at time −10 to −15 min. Only one challenge and administration of drug or combination of drugs was made in each animal.

2.1.5. Mast cell on line activation in rat mesentery detected by intravital microscopy

The rat mesentery preparation was performed according to a previous description (Guo et al., 2000). After rats were anaesthetized as mentioned above, laparotomy was performed in the supine position by midline incision and a segment of the terminal ileum was exteriorized from the peritoneal cavity. Thereafter, the animal was placed in a right semi-prone position on a heated microscope stage. Throughout the surgical procedures and experiments, the exposed tissues were superfused with a thermostated (37 °C) bicarbonate-buffered saline solution (composition in mM: NaCl 132, KCl 4.7, $CaCl_2$ 2.0, $MgSO_4$ 1.2, $NaHCO_3$ 18) equilibrated with 5% CO_2 in nitrogen to maintain physiological pH. Ruthenium Red was diluted in bicarbonate-buffered saline solution to yield a final concentration of 0.001% for mast cell staining (Shepherd and Duling, 1995; Kubes, 1999). Observation of mast cell activation (Ruthenium Red staining) in mesentery was made using a Leitz Orthoplan microscope equipped with a water immersion lens (×55, numerical apecture 0.8). The microscopic image was televised (Panasonic WV-1550, WV-1900 cameras) and recorded on videotape (Panasonic NV-F100 S-VHS recorder) for subsequent off-line analysis. Ruthenium Red (0.001%) was superfused for at least 20 min prior to treatment (time 0) and was continued throughout. Ovalbumin, dextran (Dextran-162) or compound 48/80 was administered i.v. or topically in the superfusion. The extent of degranulated mast cells was calculated by counting the number of cells stained with Ruthenium Red within a tissue area of 200 × 300 μm (0.06 mm^2) at time 0 and after 20 min of ovalbumin or compound challenge.

2.2. Compounds used

Ovalbumin (Albumin, Chicken Egg), Dextran of 162,000 average molecular weight (Dextran-162), compound 48/80, histamine, chlorpheniramine and cimetidine (Sigma, St. Louis, MO, USA) were dissolved in fresh 0.9% NaCl. When necessary, further dilutions of drugs were made in 0.9% NaCl. A stock solution of Ruthenium Red (Sigma), 1% in distilled water, was diluted in bicarbonate-buffered superfusion solution to yield a final concentration of 0.001% for superfusion.

2.3. Statistical analysis

All data are expressed as means ± S.E.M., $n = 3–6$ animals in each experimental group of rats. Two-way analysis of variance, followed by Scheffe test, was used to determine if difference between two groups at selected time point was statistically significant. A probability value of less than 0.05 was considered to be statistically significant.

3. Results

3.1. Effects of ovalbumin challenge on blood pressure and heart rate in sensitized normal +/+ and mast cell-deficient Ws/Ws rats

Ten minutes after intravenous injection of ovalbumin 1 mg kg^{-1} to the rats, mean arterial pressure fell to 43 and 51 mm Hg in sensitized +/+ and Ws/Ws rats, respec-

tively. However, no change in blood pressure was observed in sham-sensitized rats (Fig. 1A). A slight decrease in heart rate was seen only in sensitized rats (Fig. 1B).

3.2. Effects of compound 48/80 on blood pressure and heart rate in +/+ and mast cell-deficient Ws/Ws rats

Within 10 min after intravenous injection of compound 48/80 1 mg kg^{-1} to the rats, mean arterial pressure fell to 35 mm Hg in +/+ rats, but no change in blood pressure was observed in Ws/Ws rats (Fig. 2A). In the same time frame, a slight decrease in heart rate was seen in +/+ rats but not in Ws/Ws rats (Fig. 2B). The fall in mean arterial pressure was maintained throughout a 60-min observation period following compound 48/80 administration (Fig. 2A).

3.3. Effects of dextran on blood pressure and heart rate in +/+ and mast cell-deficient Ws/Ws rats

Within 10 min after intravenous injection of dextran (Dextran-162, 6%, 2 ml kg^{-1}) to the rats, mean arterial pressure fell to 55 mm Hg in +/+ rats, but no change in blood pressure was observed in Ws/Ws rats (Fig. 3A). A slight decrease in heart rate was seen in +/+ rats but not in Ws/Ws rats (Fig. 3B). The pressure recovered gradually to the normal control level by 90 min following dextran administration (Fig. 3A).

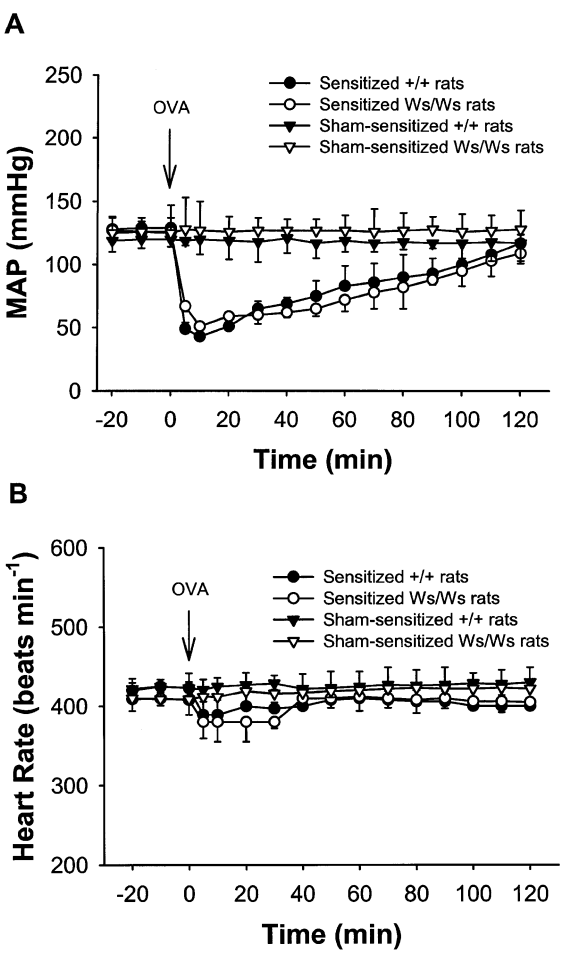

Fig. 1. Mean arterial pressure (MAP) (A) and heart rate (B) of sensitized +/+ (filled circles), mast cell-deficient Ws/Ws (open circles); and sham-sensitized +/+ (filled triangles), mast cell-deficient Ws/Ws (open triangles) rats, receiving ovalbumin (OVA, 1 g kg^{-1} i.v.) at time 0 (arrow). Data are means ± S.E.M., $n = 5$.

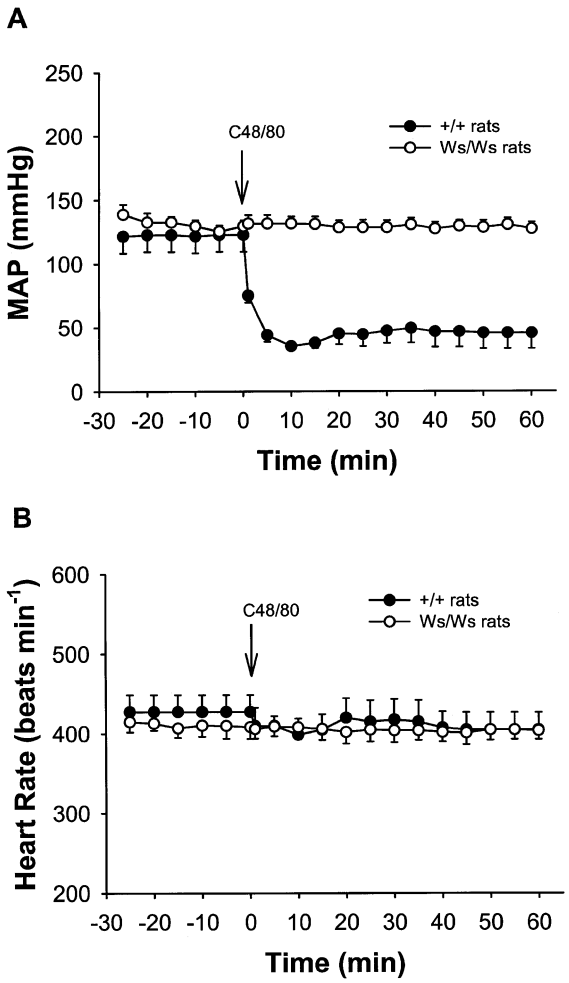

Fig. 2. Effects of compound 48/80 on mean arterial pressure (MAP) (A) and heart rate (B) in +/+ (filled circles), and mast cell-deficient Ws/Ws (open circles) rats, receiving compound 48/80 (C48/80, 1 mg kg^{-1} i.v.) at time 0 (arrow). Data are means ± S.E.M., $n = 6$.

3.4. The number of degranulated mast cells in rat mesentery after challenge with ovalbumin (i.p. or i.v.), dextran (i.v.) or compound 48/80 (i.v.)

After Ruthenium Red (0.001%) was superfused in rat mesentery for 20 min, ovalbumin (1 mg ml^{-1} at 1 mg kg^{-1}) was applied either topically or intravenously to the sensitized +/+ rats. Dextran (Dextran-162, 6%, 2 ml kg^{-1}) or compound 48/80 (1 mg kg^{-1}) was administrated intravenously in non-sensitized +/+ rats. As shown in Fig. 4, prior to any treatment there were very few mast

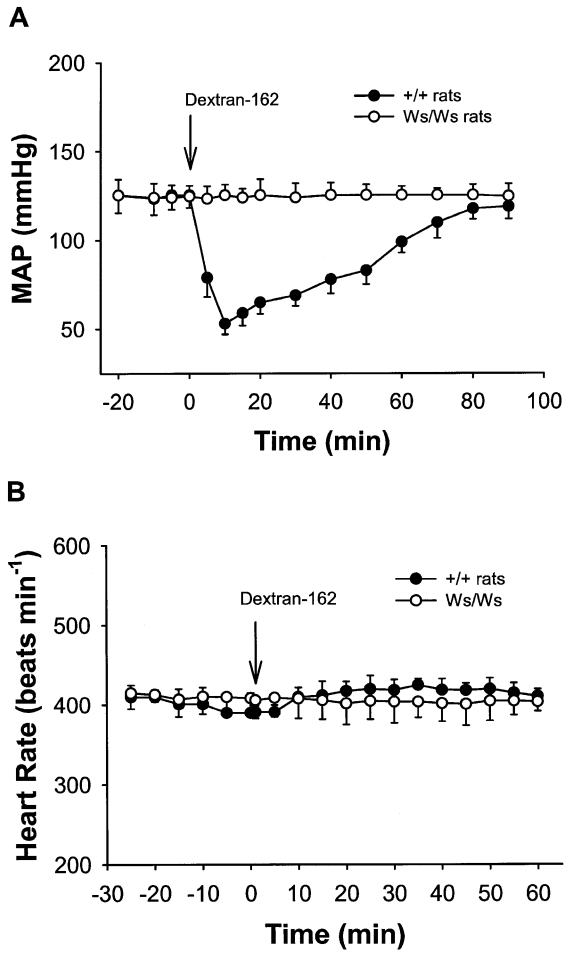

Fig. 3. Mean arterial pressure (MAP) (A) and heart rate (B) of mast cell containing $+/+$ (filled circles) and mast cell-deficient Ws/Ws (open circles) rats, receiving dextran (Dextran-162, 6%, 2 ml kg^{-1} i.v.) at time 0 (arrow). Data are means ± S.E.M., $n = 3$.

cells stained with Ruthenium Red. Many activated mast cells were detected in sensitized rat mesentery after 20 min topical challenge with ovalbumin, but not in the mesentery of sensitized animals challenged by intravenous ovalbumin. Ruthenium red stained mast cell number was increased as indication of degranulation, in both dextran and compound 48/80-treated groups 20 min after intravenous administration of these compounds (Fig. 4).

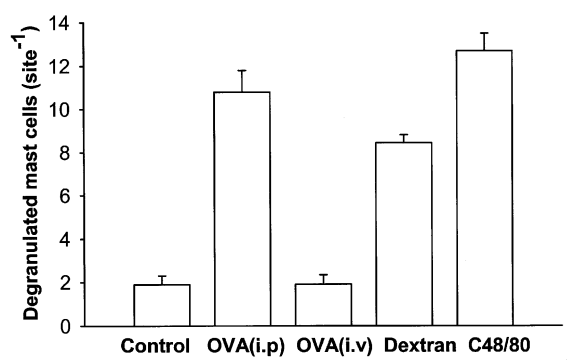

Fig. 4. Degranulated mast cell number (0.001% Ruthenium Red staining) in rat mesentery with a tissue area (site) 200×300 μm (0.06 mm^2) 20 min after receiving buffer-superfusion only (Control), ovalbumin (OVA, 1 mg kg^{-1} i.v. or i.p.) in sensitized $+/+$ rats, dextran (Dextran-162, 6%, 2 ml kg^{-1} i.v.) or compound 48/80 (C48/80, 1 mg kg^{-1} i.v.) in non-sensitized $+/+$ rats. Data are means ± S.E.M, $n = 4$.

3.5. Effect of cimetidine and chlorpheniramine on histamine-induced hypotension in $+/+$ rats

In order to determine the efficient dosage of histamine receptor antagonist for the inhibition of hypotension induced by compound 48/80 and dextran, the effects of different doses of the combination of chlorpheniramine (a histamine H_1 receptor antagonist) and cimetidine (a histamine H_2 receptor antagonist) on histamine-induced hypotension in $+/+$ rats were investigated. Moderate doses of chlorpheniramine and cimetidine (5 and 10 mg kg^{-1}, respectively) elicited approximately a 100-fold antagonism when combined (data not shown). With a high dose combination of chlorpheniramine (50 mg kg^{-1}) and cimetidine (100 mg kg^{-1}), the antagonism of histamine-induced hypotension was approximately 1000-fold (Fig. 5). This high dose combination was used in the following experiments.

3.6. Effect of histamine receptor antagonists (cimetidine and chlorpheniramine) on ovalbumin-induced hypotension in sensitized $+/+$ rats

Pretreatment with the combination of cimetidine (100 mg kg^{-1} i.v.) and chlorpheniramine (50 mg kg^{-1} i.v.) did not exert any inhibiting effect on the ovalbumin-induced hypotension in sensitized $+/+$ rats (Fig. 6). The data

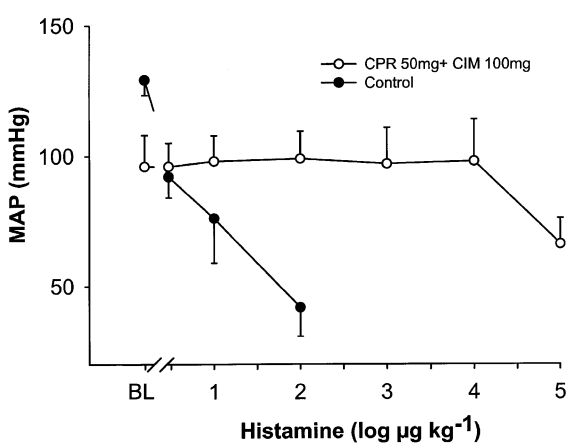

Fig. 5. Dose response of histamine (i.v.) on mean arterial blood pressure (MAP) in $+/+$ rats. The data represent histamine effects without (filled circles, control) or after 10 min pretreatment (i.v) with chlorpheniramine (CPR) 50 mg kg^{-1} together with cimetidine (CIM) 100 mg kg^{-1} (filled circles). BL represents basal level of mean arterial pressure (MAP) before administration of histamine in the absence or presence of antagonists. Data are means ± S.E.M., $n = 5$.

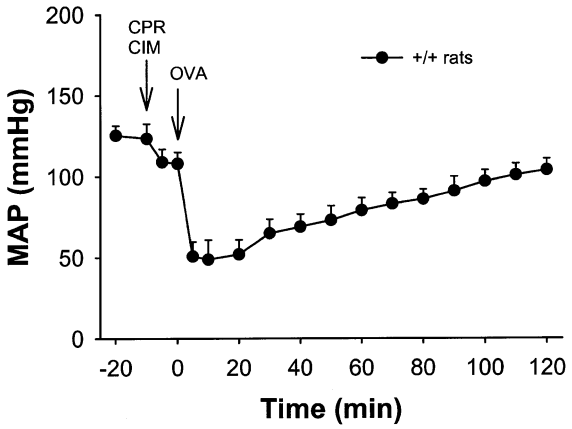

Fig. 6. Effect of combined histamine receptor antagonists on ovalbumin-induced hypotension in sensitized $+/+$ rats. Animals received histamine receptor antagonists, chlorpheniramine (CPR) 50 mg kg^{-1} and cimetidine (CIM) 100 mg kg^{-1} i.v., 10 min before ovalbumin (OVA, 1 mg kg^{-1} i.v.) challenge. Data are means ± S.E.M., $n = 6$.

suggest that mast cell amines are not involved in this response.

3.7. Effects of chlorpheniramine and cimetidine on the hypotensive response induced by compound 48/80 and dextran in +/+ rats

Pretreatment with chlorpheniramine (50 mg kg^{-1} i.v.) or cimetidine (100 mg kg^{-1} i.v.) modestly blocked the fall in blood pressure 10 min after compound 48/80 and dextran, whereas the combination of the histamine receptor antagonists caused a marked antagonism of the blood pressure lowering effect. Thus, at 10 min, mean arterial pressure was 35/55, 64/75, 78/80 (data not shown) and 107/109 mm Hg (Fig. 7), during compound 48/80/dextran alone or with cimetidine, chlorpheniramine or the combination, respectively. The data thus indicated that mast cell histamine was critically involved in the blood pressure lowering effect of compound 48/80 and dextran.

4. Discussion

The presently observed effects of antigen, dextran and compound 48/80 on blood pressure were most likely due to an effect on cardiovascular resistance vessels, and not likely due to an effect on the heart since heart rate was very little affected. An effect on blood pressure due to a change in cardiac contractility can however not be ruled out since we did not measure cardiac output.

In this study, the question of mast cell participation in systemic anaphylaxis was examined using mast cell-deficient and control rats. The results obtained do not support the view that mast cells serve as the critical effector cells in mediating the hypotensive response associated with systemic anaphylaxis since similar responses were induced in both mast cell-deficient and control rats. Mast cell responses (degranulation), above controls, could not be detected in mesentery examined from sensitized rats that had just gone into hypotension following intravenous antigen challenge. This gives support to conclusions reached by others, who have shown that mast cell-deficient mice undergo anaphylaxis (Jacoby et al., 1984; Galli and Kitamura, 1987) and that mast cell-competent mice do not require mast cell responses for anaphylaxis (Einbinder et al., 1964; Justus et al., 1990). The observation of numerous degranulating mast cells during topically applied antigen (Fig. 4) suggests that during anaphylaxis the antigen remained in the vasculature and did not reach the tissue mast cells. This means that intravascular and not extravascular cells or elements are responsible for initiating the events that give rise to the hypotensive response. In agreement, histamine receptor antagonists did not inhibit this response. In mice, anaphylaxis can be inhibited by anti-granulocyte antibody (Kimura et al., 1997). It is thus possible that mediators released not from mast cells but from other cells including neutrophils, basophils, monocytes/macrophages, platelets and endothelial cells are involved in the reaction. This would however require further investigation.

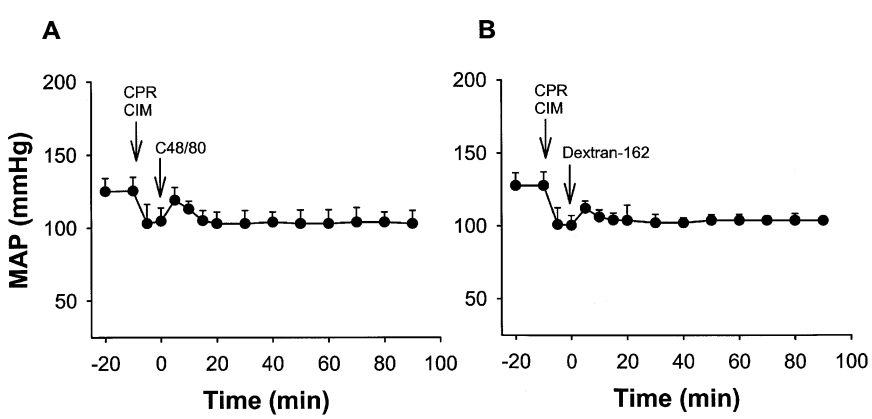

Fig. 7. Effect of as in Fig. 6 combined histamine receptor antagonists on (A) compound 48/80 (C48/80, 1 mg kg^{-1} i.v.) or (B) dextran-induced (Dextran-162, 6%, 2 ml kg^{-1} i.v.) hypotension in $+/+$ rats. Data are means ± S.E.M., $n = 6$.

In order to elucidate whether mast cell mediators were responsible for hypotension by compound 48/80 (mast cell degranulating agent) or dextran, mast cell deficient Ws/Ws rats were used. Ten minutes after i.v. injection of compound 48/80 or dextran, mean arterial pressure fell in +/+ rats. Mast cell activation (Ruthenium Red staining) was observed in rat mesentery 20 min after intravenous administration of compound 48/80 or dextran. The total lack of hypotensive effect in Ws/Ws rats demonstrates that mast cell mediators play a pivotal role in this response. We also studied animals pretreated with the combination of histamine H_1 (chlorpheniramine) and H_2 (cimetidine) receptor antagonists at doses (50 and 100 mg kg^{-1}, respectively), which exhibited a 1000-fold antagonism of the blood pressure lowering effect of exogeneous histamine. The hypotension by compound 48/80 and dextran was almost completely blocked in the drug treated group (Fig. 7). The results of our experiments agree with a previous study by Kogure et al. (1986) who showed that corticosterone inhibited dextran-induced shock in rats by inhibition of histamine release from mast cells. Our results and those of others thus indicate that mast cell histamine plays a critical role in compound 48/80 and dextran-induced hypotensive responses in rats.

The combination of histamine receptor antagonists thus caused a 1000-fold antagonism of exogenous histamine and blocked hypotension by compound 48/80 or dextran. Despite this, the antagonist combination failed to modify the hypotension induced by intravenous allergen in sensitized animals. This further supports that mast cells and histamine are not involved in the hypotensive response in systemic anaphylaxis.

Taken together, the present study suggests that active systemic anaphylaxis can be induced in mast cell-deficient rats. Mediators, not from mast cells but from unknown cells, are responsible for the hypotensive response. However, the vasoactive substances, primarily histamine, released from mast cells, play the main role in the hypotension by compound 48/80 or dextran. Genetically mast cell-deficient Ws/Ws rats, when used in comparison with normal rats, are useful and well-suited for the investigation of biological roles of mast cells.

Acknowledgements

This study was supported by grants from the Swedish Medical Research Council (projects 14X-4342, 04P-10738 and 04X-7919), the Swedish Foundation for Health Care Sciences and Allergy Research (A98110), Lars Hiertas Minne, and Karolinska Institute.

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