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Inhibition of mast cell degranulation-induced drop of blood pressure with clemastine, cromolyn and compound 48/80 pretreatment

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Introduction

Many basic compounds, including numerous drugs, can release histamine and other inflammatory mediators from mast cells [1]. Drug-induced degranulation of mast cells is an undesired effect and therefore should be avoided. One of the consequences of drug-induced mast cell degranulation is a drop of the mean arterial blood pressure (MAP) [2]. Prevention of the drop of MAP with specific agents, targeting either the action of released mediators from mast cells or the extent of mast cell degranulation, is a reasonable approach to clarify the mechanism of this undesired effect. In this study we evaluated three in vivo models using this approach. In all three models compound 48/80 was used as a tool to mimic the undesired mast cell degranulation effect of potential new drugs and the drop of MAP was measured following its application. In the first two models inhibition of the drop of MAP with the selective H1 antagonist clemastine and with the mast cell stabilizer cromolyn was determined respectively. In the third model the drop of MAP was studied in mast cell depleted rats.

Materials and methods

Mean arterial pressure measurements

Male Wistar rats (370–500 g) were anaesthetized with urethane (1.4 g/kg, intraperitoneally), according to local ethical committee guidelines. Millar SPR-407 pressure transducer was introduced through the right femoral artery up to the abdominal aorta. The arterial pressure signal was continuously recorded using Dewesoft 6.1b14 acquisition software and processed using FlexPro 6.0.22 software.

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Study design

In all treatment groups MAP recording started at t = 0 min.

To determine the effect of clemastine on the MAP response induced by compound 48/80, clemastine (0.13 mg/kg/min, 15 min; n = 4) or saline (n = 4) were infused intravenously, starting at t = 5 min. Following infusion, at t = 20 min, compound 48/80 (0.3 mg/kg) was injected intravenously.

To determine the effect of cromolyn on the MAP response induced by compound 48/80, cromolyn (10 mg/kg, n = 4) or saline (n = 4) were injected intravenously at t = 5 min, immediately followed by intravenous injection of compound 48/80 (0.3 mg/kg).

Mast cell depletion can be achieved by repeated administration of compound 48/80 [3]. Therefore, in the model using this approach, rats were pretreated once per day subcutaneously for three consecutive days with compound 48/80 (5 mg/kg; n = 4) or saline (n = 4). On the 4th day of the experiment compound 48/80 (0.3 mg/kg) was injected intravenously at t = 5 min.

Statistically significant differences between treatment groups were determined by repeated measures ANOVA (Graph Pad Prism 4.02). A value of $p < 0.05$ was considered significant. All data are given as mean ± standard error of mean (S.E.M.).

Results and discussion

In agreement with the previous studies [2, 4], compound 48/80-induced mast cell degranulation resulted in a sudden drop of MAP immediately after intravenous administration (Fig. 1). This effect was partially inhibited with clemastine pretreatment ($p = 0.006$) (Fig. 1). A complete inhibition was not achieved, because other released mediators could also contribute to a drop of MAP [5, 6]. Another plausible cause for incomplete inhibition with H1 selective antagonist clemastine could be the fact that H2 receptors are also involved in the control of MAP via vasodilatation [7].

Pretreatment of rats with cromolyn resulted in a significant inhibition of compound 48/80-induced drop of MAP ($p = 0.012$) (Fig. 1). However, as with the clemastine pretreated group, complete inhibition against compound 48/80-induced

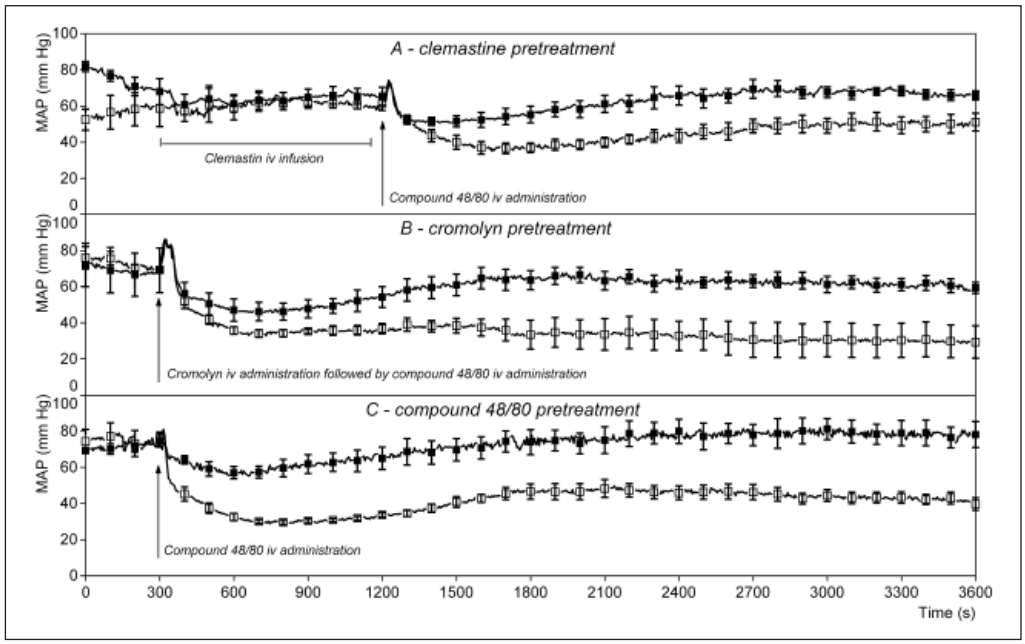

Fig. 1. Effect of compound 48/80 (0.3 mg/kg) on mean arterial blood pressure (MAP) in rats pretreated with (*A*) clemastine (0.13 mg/kg/min, 15 min, infusion, ■) and saline (infusion, □); (*B*) cromolyn (10 mg/kg, intravenous, ■) and saline (intravenous, □); (*C*) compound 48/80 (5 mg/kg/day, 3 days, subcutaneously, ■) and saline (3 days, subcutaneously, □). Results are expressed as mean ± S.E.M. of four rats.

drop of MAP was not observed. This result was unexpected, since it has previously been reported that cromolyn pretreatment resulted in a complete inhibition of compound 48/80-induced drop of MAP [2]. This discrepancy could be explained by the different doses of compound 48/80 and cromolyn used.

In the third model compound 48/80 evoked only a minor drop of MAP in mast cell depleted rats (Fig. 1), which was significantly smaller than in the control (saline pretreated) group ($p = 0.006$). A minor drop of MAP, however, was still observed, probably due to the incomplete degranulation of mast cells in the pretreatment period [3, 6].

In conclusion, we evaluated a method to determine if the undesired drop of MAP, caused by potential new drugs, is related to mast cell degranulation. All three models described herein could be used to study drug-induced drop of MAP, particularly in cases where drug-induced mast cell degranulation is suspected.

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