Pharmacia–JTPA
26: 57(3), 131–137, 1986

ANTIBRADYKININ AND ANTIHISTAMINIC ACTIONS OF HALOPERIDOL, CHLORPROMAZINE AND TRIFLUOPERAZINE-I: STUDIES ON THE ISOLATED GUINEA - PIG ILEUM

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SUMMARY:

Antibradykinin and antihistaminic effects of haloperidol, chlorpromazine and trifluoperazine had been previously reported. Since these effects which are not fully understood in terms of their mechanisms might be clinically, important it is aimed to investigate mentioned effects on the different preparations. The present study is designed to characterize their effects on isolated guinea-pig ileum.

Haloperidol inhibited the bradykinin- and histamineinduced responses on the guinea-pig ileum in a non-competitive manner. While chlorpromazine and tirfluoperazine inhibited the bradykinin-induced responses non-competitively, the histamine-induced responses were abolished in a quite different manner. It is observed that the inhibition of the histamine-induced responses is either competitive or non-competitive depending on the concentrations of the phenothiazines.

HALOPERİDOL, KLORPROMAZİN VE TRİFLUOPERAZİN'İN ANTİBRADİKİNİN VE ANTİHİSTAMİNİK ETKİLERİ I: İZOLE KOBAY İLEUMU ÜZERİNDEKİ ÇALIŞMALAR

ÖZET:

Haloperidol, klorpromazin ve trifluoperazin'in antibradikinin ve antihistaminik etkileri daha önceden bildirilmişti. Fakat tam olarak mekanizmaları anlaşılamayan bu etkiler klinik açıdan önemli olabilir. Bahsedilen bu etkilerin çeşitli preparatlarda inceienmesi amaçlanmıştır. Bu çalışma ile izole kobay ileumda etkilerin belirlenmesi düşünülmüştür.

Haloperidol, kobay ileumunda bradikinin ve histaminin indüklenmiş cevaplarını kompetitif olmayan şekilde inhibe etmiştir. Klorpromazin ve trifluoperazin de indüklenmiş bradikinin cevaplarını inhibe etmişler, fakat indüklenmiş histamin cevabı daha değişik bir tarzda inhibe edilmiştir.

İndüklenmiş histamin cevapların kompetitif veya kompetitif olmayan şekilde inhibe edilmesi fenotiyazinin konsantrasyonuna bağlıdır.

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INTRODUCTION

Bradykinin and histamine are endogenous substances which are involved in a variaty of pathophysiological conditions, e.g. anaphylaxis, acute inflammation, shock, acute pancreatitis etc. Histamine contracts many smooth muscles, such as those of the bronchi and gut(1,2), but powerfully relaxes others, including those of fine blood vessels(3). It is also a potent stimulus to gastric acid production(4) and elicites other exoorine secretions(5). Some of these effects, such as bronchoconstriction and contraction of the gut, are readily antagonized by the long-available antihistamines such as promethazine, diphenhydramine etc., and are considered to involve H_1 receptors(6). Others, most notably gastric secretion, are completely refractory to such antagonists, involve activation of H_2 receptors, and are susceptible to inhibition by cimetidin and related drugs(7).

Bradykinin possesses an extraordinarily high degree of pharmacological activity. In extremely low doses it causes vasodilatation, increases capillary permeability and produces edema, evokes pain by some effects on nerve endings, and contracts or relaxes a variety of extravascular muscles(8,9). Unfortunately, there is no specific antagonist for the actions even though some convincing investigations have being performed to discover a clinically useful antibradykinin agent(10,11). It is known that several compounds, such as phetothiazine derivatives inhibit certain effects of bradykinin non-specitically (12,13,14,15,16). In addition, chlorpromazine has been found to be as an inhibitor of 5-hydroxytrayptamine(17). On the other hand, haloperidol is an antibradykinin agent with respect to hypotensive action of this peptide(18). Recent investigations indicate that haloperidol and chlorpromazine potentiate the prostaglandin-induced contraction on the rat stomach fundus strips(19).

In this study, it is aimed to investigate possible interactions of haloperidol, chlorpromazine and trifluoperazine with bradykinin and histamine, and examine the specificty of these interactions which were also previously shown on the some other preparations.

METHODS

Isolated Guinae-Pig Ileum

The isolated guinea-pig ileum was prepared according to the method described by(20). Male and female guineapigs, weighing 250 to 400 g, were killed by a blow on the head and exanguinated. The terminal ileum pieces (2 cm) were obtained and suspended in a 10-ml organ bath filled with Tyrode solution (in mmol: NaCl 140, KCl 2.7, $CaCl_2$ 1.36, $MgCl_2$ 0.53, $NaHCO_3$ 12, Na_2HPO_4 0.36 and glucose 5.6) at 37°C and gassed with 5 per cent CO_2 in oxygen.

The contractions of the isolated guinea-pig ileum were recorded using an isotonic transducer (Ugo Basile, No: 7006) connected to a recording microdynamometer (Ugo Basile, No: 7050). The load on the tissue was 1.0 g. The contractions were magnified 5-folds.

The suspended ileum was allowed to equilibrate for 60 minutes, in while the fluid of the bath was rinsed every 10 minutes. After this initial incubation, non-cumulative doseresponse curves were obtained for an agonist using two individual dose-response procedure in all experiments. The agonists were histamine and bradykinin. In each experiment, only one concentration of an antagonist was tested. The drugs used as antagonists were

haloperidol, chlorpromazine and trifluoperazine. The isolated guinea-pig ileum was incubated with these drugs for 10 minutes and then the same dose-response procedure was repeated.

Analysis of Data

To evaluate the actions of antagonists on the guinea-pig ileum, pD'_2 values were calculated(21).

All values reported represent the results of individual experiments. The dose-response curves obtained in the presence and in the absence of the antogonist were analysed by means of linear regression. When indicated, significance of differences between the mean values was determined by the Student's t-test(22).

Drugs used

Bradykinin triacetate (Sigma), Chlorpromazine hydrochloride (Eczacıbaşı-Turkey), Haloperidol hydrochloride (Zaman Ecza-Turkey), Histamine diphosphate (Sigma), Trifluoperazine dihydrochloride (Dr.F.Frik-Turkey). All dilutions were prepared with fresh Tyrode solutions.

RESULTS

Haloperidol, in a concentration range of $2.2x10^{-6}$ - $8.8x10^{-6}$M, inhibited the contractions elicited by bradykinin and histamine non-competitively (Figure 1) Chlorpromazine ($2.5x10^{-7}$M) and trifluoperazine ($6.0x10^{-8}$ - $1.2x10^{-7}$M) inhibited bradykinin-induced contractions of guinea-pig ileum, non-competitively (Figure 2), while histamine-induced contractions were inhibited by these phenothiazines in a quite different manner (Figure 3). In the dose ranges of $5.0x10^{-11}$ to $5.0x10^{-9}$M and $1.2x10^{-11}$ to $1.2x10^{-9}$M, chlorpromazine and trifluoperazine inhibited the contractions elicited by histamine. However, over the certain concentrations (for those of chlorpromazine: $5.0x10^{-7}$M and of trifluoperazine: $1.2x10^{-7}$M), these inhibitions were non-competitive in nature. The pD'_2 and pA_2 values concerning all these inhibitions are summarized on Table I.

Table 1. Competitive and Non-competitive affinity constants of Haloperidol, Chlorpromazine and Trifluoperazine on the guinea-pig isolat deileum.

	Bradykinin	Histamine	
Antagonists	pD'_2 + SEM	pD'_2 + SEM	pA_2 + SEM
Haloperidol	5.36 + 0.24	5.56 + 0.19	—
Chlorpromazine	6.48 + 0.16	6.89 + 0.22	9.78 + 0.15
Trifluoperazine	6.97 + 0.18	7.73 + 0.02	9.39 + 0.21

pD'_2 : Non-competitive antagonist affinity constant,

pA_2 : Competitive antagonist affinity constant,

SEM : Standart error of mean (n = 6).

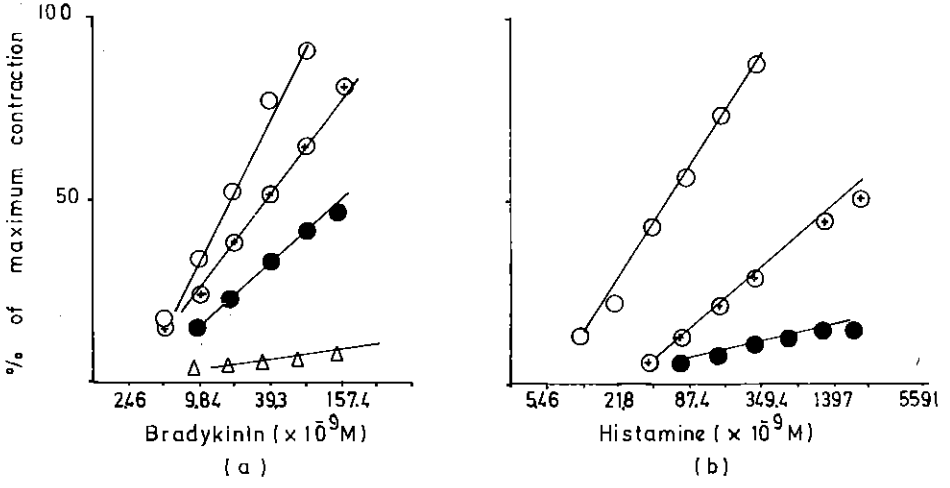

Figure 1. Non-competitive inhibition of bradykinin (a) and histamine (b) by haloperidol on the guinea-pig ileum. (○) Dose-response curve of bradykinin (a), and (○) histamine (b) (n:30, n:25 respectively), (⊕) In the presence of 2.2×10^{-6}M haloperidol (n:9, n:6 respectively), (●) 4.4×10^{-6}M haloperidol (n:6, n:5 respectively), (△) 8.8×10^{-6}M haloperidol (n:4).

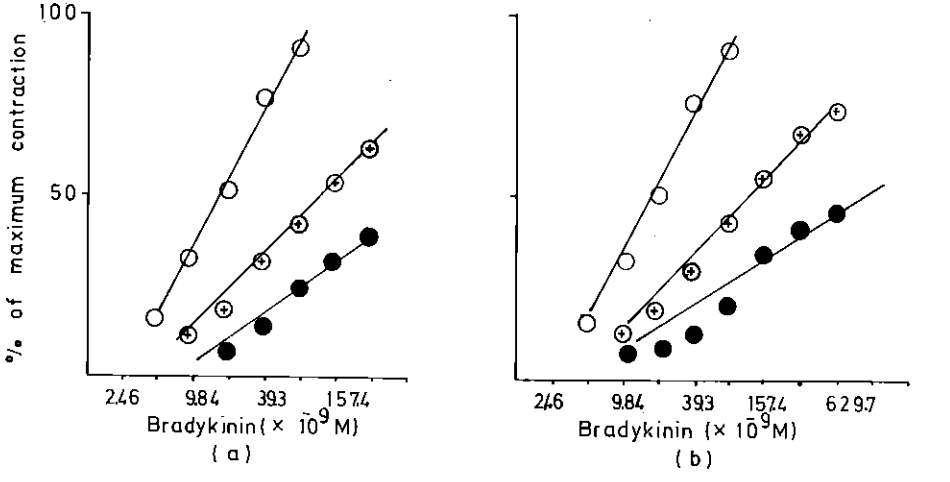

Figure 2 Non-competitive inhibition of bradykinin by chlorpromazine (a) and trifluoperazine (b) on the guinea-pig isolated ileum. (○) dose response curve of bradykinin (n:25), (⊕) in the presence of 2.5×10^{-7}M chlorpromazine (a) and 6.00×10^{-8}M trifluoperazine (b) (n:6, n:7 respectively), (●) dose-response curves of bradykinin in the presence of 5.00×10^{-7}M chlorpromazine (a) and 1.2×10^{-7}M trifluoperazine (b) (n:7, n:5, respectively).

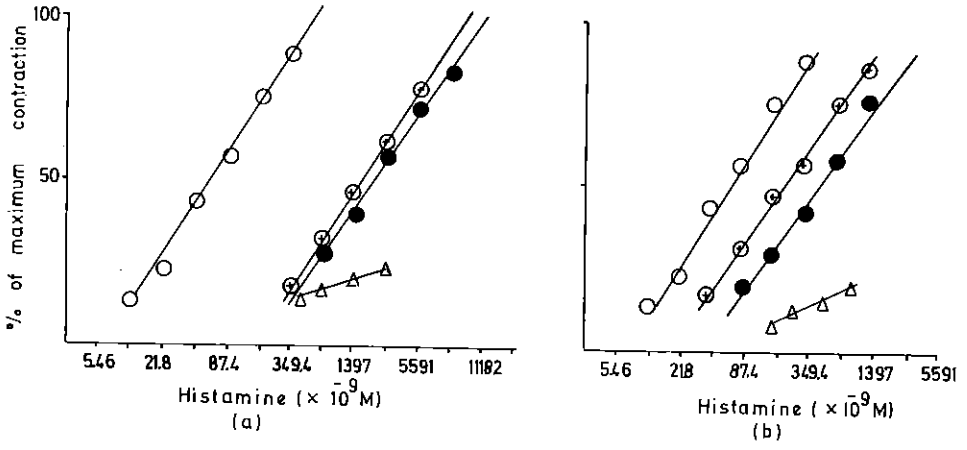

Figure 3 Inhibition of histamine by chlorpromazine (a) and trifluoperazine (b) on the guinea-pig isolated ileum. (○) Dose-response curve of histamine (n:27), (⊕) in the presence of $5.00x10^{-11}$M chlorpromazine (a) and $1.2x10^{-11}$M trifluoperazine (b) (n:7), (●) $5.00x10^{-9}$M chlorpromazine (a) and $1.2x10^{-9}$ M trifluoperazine (b) (n:8), (△) $5.00x10^{-7}$M chlorpromazine (a) and $1.2x10^{-7}$M trifluoperazine (b) (n:6, n:4 respectively.)

DISCUSSION

One of the purposes of this study was to examine possible interactions of haloperidol, chlorpromazine and trifluoperazine with bradykinin and histamine on the gut. The findings obtained in this study pointed out that haloperidol, chlorpromazine and trifluoperazine possess antihistaminic and antibradykinin properties on the guinea-pig ileum. At this point, several questions might be addressed in relation with these properties of haloperidol, chlorpromazine and trifluoperazine; 1- What is the specificity of these antihistaminic and antibradykinin actions? 2- Where are the sites of action of these compounds on the smooth muscle cells?

Since the bradykinin- and histamine-induced contractions of guinea-pig ileum were inhibited by haloperidol non-competitively, it is considered that the haloperidol-induced inhibitions are due to non-specific interactions on the smooth muscle cells. Confirmatively, it has been demonstrated that haloperidol inhibits substance P-induced contractions on the isolated guinea-pig ileum non-competitively and antagonized bradykinin-induced hypotension(18). Under the light of recent investigations, it has been welldocumented that haloperidol exerts calmodulin-blocking actions on various preparations, such as bovine brain homogenates(23,24). For that reason, calmodulin blockade seems like a responsible mechanism for the antihistaminic and antibradykinin actions of haloperidol on the guinea-pig ileum.

Chlorpromazine and trifluoperazine are also considered as non-specific agents because of the non-competitive inhibition of the bradykinin action on the guinea-pig ileum. There have been many studies of the antibradykinin effects of phenothiazines on various preparations confirming our results. For example, bradykinin-induced writhing response on mice(12), pressor action of bradykinin on human placental circulation(25) and contractile action on the guinea-pig ileum(13,15) have been found to be inhibited by pheno-

thiazines in different degrees. The responsible mechanism for the non-specific inhibitory actions of chlorpromazine and trifluoperazine might be calmodulin blockade. In fact, these phenothiazine derivatives are able to inhibit calmodulin on smooth(26)

In our experiments, we observed that chlorpromazine and trifluoperazine possess two different types of action on the contraction of guinea-pig ileum elicited by histamine. First, chlorpromazine (in a concentration range of $5.0x10^{-11}$ to $5.0x10^{-9}$M) and trifluoperazine (in a concentration range of $1.2x10^{-11}$ to $1.2x10^{-9}$M) inhibited the histamine-induced contractions in competitive manner suggesting specific antihistaminic action via H_1 receptors. It is interesting that this competitive inhibitions appear in extremely low concentrations of chlorpromazine and trifluoperazine. As a matter of fact, it is well-known that chlorpromazine and its congeners possess different degrees of antihistaminic actions through H_1 receptors. In fact, promethazine is a clinically useful antihistaminic in the treatment of various allergic states.

Second, chlorpromazine and trifluoperazine (at the concentrations of $5.0x10^{-7}$M and $1.2x10^{-7}$M, respectively) antagonized the contractions of guinea-pig ileum elicited by histamine in non-competitive manner. These inhibitions strongly suggest that the phenothiazine derivatives have a non-specific action on the smooth muscle cells. As seen in Figure: 3 the inhibition profiles chlorpromazine and trifluperazine are closely similar. Therefore, it seems likely that the non-competitive inhibitions of the histamine-induced contractions as well as the bradykinin-induced contractions by these phenothiazines occur through a common mechanism. Confirmatively, it has been shown that trifluoperazine inhibits the calcium-induced contractions on the K^+ -depolarized rat duodenum in a non-competitive way(27).

Under the light of this study, it is understood that chlorpromazine and trifluoperazine possess non-specific antibradykinin and specific + non-specific antihistaminic effects, whereas haloperidol has only non-specific antibradykinin and antihistaminic effects on the isolated guinea-pig ileum. The non-specific actions of these agents might be due to calmodulin blockade, since the concentrations of these phenothiazine derivatives in which the non-competitive inhibitions against histamine and bradykinin occured are almost the same as the concentrations required for calmodulin inhibition(23). Thus, it would be of interest to examine these compounds on the other preparations.

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