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SPECIAL REPORT

Effects of WIN 64338, a nonpeptide bradykinin B_2 receptor antagonist, on guinea-pig trachea

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We investigated the effect of the nonpeptide bradykinin receptor antagonist, [[4-[[2-[[*bis*(cyclohexylamino)methylene] amino]-3-(2-naphthalenyl) 1-oxopropyl]amino]-phenyl]-tributyl, chloride, monohydrochloride (WIN 64338), on [^{3}H]-bradykinin binding and on bradykinin-induced contraction of the guinea-pig trachea. This non peptide bradykinin receptor antagonist inhibited [^{3}H]-bradykinin binding with a nanomolar range of affinity, $K_i = 50.9 \pm 19$ nM and inhibited bradykinin-induced contraction in a non-competitive manner with a K_B value of 6.43 $10^{-8} \pm 2.34$ 10^{-8} M.

Keywords: Bradykinin; nonpeptide bradykinin receptor antagonist; trachea; bradykinin binding

Introduction Bradykinin (BK) is a natural occurring inflammatory nonapeptide which is generated by cleavage of high and low molecular weight kininogens by tissue or plasma kallikreins. Acting on two types of receptors, B_1 and B_2, bradykinin possesses a wide range of actions. Following the discovery of the first bradykinin B_2 receptor antagonists by Vavrek & Stewart (1985), a number of other antagonists have been synthesized and tested *in vitro* and *in vivo*. A number of these B_2 receptor antagonists showed some limitations since they were partial agonists in various systems or displayed some affinity for B_1 receptors after degradation by kininase I. New more potent bradykinin B_2 receptor antagonists have recently been described such as D-Arg-[Hyp^3, Thi^5, D-Tic^7, Oic^8]-bradykinin (Hoe 140) (Wirth *et al.*, 1991), D-Arg^0[Hyp^3, D-HypE(*trans*-propyl)^7, Oic^8]bradykinin (NPC 17731) and D-Arg^0 [Hyp^3, D-HypE(*trans*-thio-phenyl)^7, Oic^8]bradykinin (NPC 17761) (Kyle *et al.*, 1991). We have tested all these compounds on the bradykinin-induced contraction of the guinea-pig trachea, and NPC 17761 appears to be the most interesting antagonist since it inhibited BK-induced contraction in a competitive manner and was devoid of any agonist activity (Trifilieff *et al.*, 1992; 1993). However, these three compounds were all peptidic antagonists. Recently, the first nonpeptide B_2 receptor antagonist, [[4-[[2-[[*bis*(cyclohexylamino)methylene] amino]-3-(2-naphthalenyl) 1-oxopropyl]amino]-phenyl]-tributyl, chloride, monohydrochloride (WIN 64338), was investigated in human lung fibroblasts and guinea-pig ileum (Sawutz *et al.*, 1993; Salvino *et al.*, 1993) and it has been proposed that it is ileal-selective (Farmer & DeSiato, 1994). In this study we investigated the effect of this nonpeptide antagonist on bradykinin binding and on bradykinin-induced contraction of guinea-pig trachea.

Methods *Competition of [^{3}H]-bradykinin binding* Membrane preparations, displacement of specific [^{3}H]-bradykinin in epithelium denuded tracheal or ileum membranes and determination of K_i values were performed as described previously (Trifilieff *et al.*, 1992). *n* reflects the number of experiments performed on separate membrane preparations from two or three guinea-pigs.

Isolated trachea studies Tracheal strips denuded of epithelium were prepared (Trifilieff *et al.*, 1992) and suspended in 10 ml organ baths at 37°C. After 3 washes at 15 min intervals and equilibration at a baseline tension of 2 g, variation in smooth muscle tone was measured isometrically. DL-Thiorphan (10^{-5} M) and the antagonist, WIN 64338, were added 30 and 20 min respectively before the first dose of bradykinin. The concentration-response relationship for BK, in the presence or absence of WIN 64338, was determined with a cumulative dose schedule (10^{-10} to 10^{-5} M). At the end of the experiment, carbachol (10^{-3} M) was added as a control. The agonist activity of WIN 64338 was tested with a cumulative dose schedule (10^{-10} to 10^{-5} M). Concentration-response curves for BK and for inhibition of BK-induced contraction by WIN 64338 were carried out on different tracheal rings belonging to the same animal. The K_B value of WIN 64338 has been determined with a double-reciprocal plot of equieffective concentrations of agonist (A) in the absence (1/A) and in the presence (1/A') of WIN 64338. K_B was derived from the equation $K_B = [B]$/slope-1 (Kenakin, 1993a). All data are expressed as the means ± s.e.mean and Student's *t* test for paired samples was used to determine the significance of the difference between mean values in all control and test tissues. n represents the number of experiments for each point of each curve.

Drugs [^{3}H]-bradykinin (106 Ci mmol^{-1}) was obtained from New England Nuclear (Boston, MA, U.S.A.). Unlabelled bradykinin was purchased from Sigma Chemicals Co. (St. Louis, MO, U.S.A.). WIN 64338 was a gift from Sanofi Winthrop (Collegeville, PA, U.S.A.).

Results *Competition experiments* In competition experiments WIN 64338 inhibited [^{3}H]-bradykinin (0.5–0.6 nM) binding with K_i values of 50.9 ± 19 nM ($n = 3$) in epithelium denuded tracheal membrane preparations and 34.1 ± 1.7 nM ($n = 3$) in ileum membrane preparations while, unlabelled bradykinin yield K_i values of 203.1 ± 51 pM and 304.3 ± 35 pM respectively. The K_i values for unlabelled bradykinin are in agreement with our previous study (Trifilieff *et al.*, 1992).

Isolated trachea Bradykinin induced a dose-dependent contraction of the epithelium-denuded guinea-pig trachea with an EC_{50} value of 2.2 ± 0.7 nM and a maximal contraction of 1598 ± 130 mg. WIN 64338 antagonized the bradykinin-induced contraction in a non-competitive manner (Figure 1), the Schild plot slope being 1.35 ± 0.36 and the K_B value being 6.43 $10^{-8} \pm 2.34$ 10^{-8} M. The compound was devoid of agonist activity up to 10^{-5} M. In addition, WIN 64338 did not affect contractility non-specifically since it did not affect the contraction induced by carbachol.

Discussion A great number of bradykinin analogues have been synthesized in the search for receptor antagonists for

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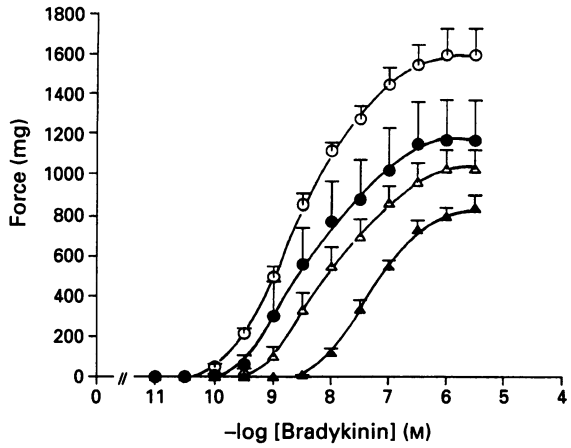

Figure 1 Log concentration-response curves for bradykinin in guinea-pig trachea in the absence (○) and presence of the B_2 receptor antagonist, WIN 64338: (●) 100 nM; (Δ) 300 nM; (▲) 1000 nM. $n=8$ for control and treated trachea.

bradykinin. The first antagonists, [D-Phe^7]-substituted bradykinins (Vavrek & Stewart, 1985) have been shown to have limited action since they were partial agonists in various systems and, following degradation by kininase I, displayed some affinity for B_1 receptors. The development of other analogues substituted at positions 7 and 8 has resulted in potent long-lasting bradykinin receptor antagonists (Wirth *et al.*, 1991). Several of these novel peptides, including Hoe 140, NPC 17761 and NPC 17731 antagonize bradykinin-induced responses of guinea-pig trachea (Trifilieff *et al.*, 1992; 1993). This study, performed in guinea-pig trachea, was undertaken to establish the type of antagonism exhibited by the first non-peptide bradykinin antagonist. The present results conflict with those of Farmer & DeSiato (1994) who reported that WIN 64338 was inactive in the guinea-pig trachea. The reasons for these contrasting results is unknown but our results demonstrated that this compound possesses similar binding affinities in guinea-pig trachea and ileum, the latter being recognized as a B_2 tissue source. The fact that WIN 64338 inhibited BK-induced contraction in the guinea-pig trachea in a non-competitive manner (present study) whereas it has been reported as a competitive antagonist in the guinea-pig ileum (Farmer & Desiato, 1994) is difficult to explain. However it must be kept in mind that kinins exert complex indirect effects in the guinea-pig trachea, therefore it is still possible that WIN 64338 is a competitive antagonist because a competitive antagonist may produce a decrease in E_{max} and a loss of parallelism to an indirect agonist (Kenakin, 1993b). The present results together with our previous studies (Trifilieff *et al.*, 1992; 1993) led us to suggest that bradykinin receptors of the guinea-pig trachea are similar to those present in the guinea-pig ileum and belong to the B_2 subtype.

In conclusion, this study showed that WIN 64338 is the first non-peptide B_2 antagonist which is able to inhibit the bradykinin-induced contraction in the epithelium-denuded guinea-pig trachea.

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