Pulmonary, gastrointestinal and urogenital pharmacology

Characterization of ibodutant at NK₂ receptor in human colon

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1. Introduction

Tachykinin receptors, namely NK₁, NK₂ and NK₃, are seven transmembrane class-A (rhodopsin-like) G-protein coupled receptors, widely expressed in the central and peripheral mammal tissues (Shimizu et al., 2008). In the mammalian intestine, tachykinins play a role as excitatory transmitters that mediate the ascending excitatory reflex and atropine-resistant peristalsis (Barthó and Holzer, 1985; Holzer and Holzer-Petsche, 1997a). In the human colon, the tachykinin receptors mediating smooth muscle contraction belong, for the most part, to the NK₂ type (Maggi et al., 1993) although radioligand binding studies (Warner et al., 1999) have revealed the presence of a small population of NK₁ receptors, whose functional role remains to be elucidated.

In this view, tachykinin NK₂ receptor antagonists are regarded as possible candidates for counteracting altered smooth muscle motility and visceral hypersensitivity present in pathological conditions characterized by an inflammatory state and impaired motility such as irritable bowel syndrome (IBS) (Lecci et al., 2004 for review), and represent potential innovative therapeutic drugs (Lecci et al., 2006; Quartara et al., 2009).

We recently developed a new potent tachykinin NK₂ receptor-selective nonpeptide antagonist, ibodutant (previously named MEN15596) that is presently undergoing Phase-IIb clinical trial for treatment of diarrhea-predominant IBS. The pharmacological outlines of ibodutant have indicated its high affinity and selectivity for the human tachykinin NK₂ receptor over the NK₁ and NK₃, and subnanomolar antagonist potency in human, guinea-pig, and minipig in vitro bioassays (Cialdai et al., 2006). Moreover, ibodutant displays a long duration of action both in vivo and in vitro, due to its bioavailability, metabolic resistance, and long residence...
time on the tachykinin NK2 receptor (Cialdai et al., 2006; Meini et al., 2009).

Smooth muscle preparations of the human colon have been previously used to characterize both the potency and affinity of different tachykinin NK2 receptor antagonists (Giuliani et al., 1991; Advenier et al., 1992; Warner et al., 1999; Patacchini et al., 2000). Therefore, the present investigation was undertaken to characterize the pharmacological properties of ibodutant at the tachykinin NK2 receptor in the circular muscle of the human colon. Radioligand binding experiments using iodinated neurokinin-A (NKA) and smooth muscle membranes were performed to assess ibodutant affinity in comparison to that of other tachykinin NK2 receptor antagonists (nepadutant and saredutant, formerly known as MEN11420 and SR48968; Catalioto et al., 1998; Emonds-Alt et al., 1993). Smooth muscle contractility experiments were performed to evaluate the ibodutant antagonist potency and the reversibility of receptor blockage towards the responses produced by the tachykinin NK2 receptor selective agonist [\(\beta\)Ala\(^8\)NKA(4-10)]. Moreover, since some sex-related variations in NK2 receptor pharmacology in human colon have been described (Burcher et al., 2008) we assessed whether the response to NK2 receptor agonist and antagonist differs, at some extent, in colonic strips from male and female patients.

2. Materials and methods

2.1. Patients and specimens

All the procedures used in the present study were approved by the Ethics Committee of the Medical Faculty of Florence University. Written informed consent was obtained from all patients. Segments of human colon, approximately 10 cm in length, were taken from grossly normal margins of surgical resections from 16 patients (7 males and 9 females, age range 47–84 years) undergoing partial colectomy for adenocarcinoma. Most segments were taken from the descending colon (9) and some from sigmoid (3), ascending (2) and transverse (2) colon.

Immediately after resection, colonic segments were placed in ice-cold Ringer-lactate solution and quickly transported to the laboratory. No patient received radio or chemotherapy before surgery. All the procedures used in the present study were approved by the Ethics Committee of the Medical Faculty of Florence University.

The tissue was transferred into fresh oxygenated (95% O\(_2\) and 5% CO\(_2\)) ice-cold Krebs solution of the following composition (mmol/l): NaCl 119; NaHCO\(_3\) 25; KH\(_2\)PO\(_4\) 1.2; MgSO\(_4\) 1.5; CaCl\(_2\) 2.5; KCl 4.7 and glucose 11, cleaned from serosal fat, mucosal layer and tenia coli leaving the smooth muscle bands. Part of this muscle was weighed, frozen and stored in liquid nitrogen and thawed on ice and then placed in 15 volumes of 50 mM Tris HCl (pH 7.4) containing 120 mM NaCl and 5 mM KCl (pH 7.4). It was first minced by using fine scissors and then homogenized by using a homogenizer (Ultra-Turrax T25, IKA Labortechnik) set at 20,500 loads min\(^{-1}\). The homogenate was centrifuged at 48,000 g for 20 min and the pellet was re-homogenized and re-suspended in 50 mM Tris HCl (pH 7.4) containing 10 mM EDTA and 300 mM KCl for 1 h. After centrifugation as before, the membranes were washed twice in 50 mM Tris HCl. Before the last centrifugation the protein content was determined according to Bradford (1976). The membranes were finally re-suspended in binding buffer.

2.4. Radioligand binding studies

The binding buffer was 50 mM Tris HCl (pH 7.4) containing 0.02% bovine serum albumin (BSA), 3 mM MnCl\(_2\), 100 mg/ml bacitracin, 10 mg/ml chymostatin, 5 mg/ml leupeptin, and 2.5 mg/ml thiorphan. Non-specific binding was defined as the amount of radio-labelled ligand bound in the presence of unlabeled NKA (1 \(\mu\)M). Preliminary experiments were performed to verify the protein concentration to be used (about 70–100 mg/ml, final concentration) and the time to reach the radioligand binding steady state (60 min). Competing ligands (NKA, ibodutant, saredutant and nepadutant) were tested in a wide range of concentrations (1 pM–10 \(\mu\)M, and serial dilutions were performed with binding buffer. \([\text{\textsuperscript{125}}\text{I}]\)NKA concentration was in the range between 0.066 and 0.093 nM. Binding reaction (at room T) started at the time of membranes addition (final volume of 500 \(\mu\)l) and stopped 60 min later by rapid filtration through UniFilter-96 plates (Packard Instrument Company), pre-soaked overnight in BSA 0.5%, and using a MicroMate 96 Cell Harvester (Packard Instrument Company). The tubes and filters were then washed five times with 0.5 ml aliquots of Tris buffer (50 mM, pH 7.4, 4 \(^\circ\)C) containing 3 mM MnCl\(_2\) and 0.02% BSA. Filters were dried and soaked in Microscint 40 (50 \(\mu\)l/well, Packard Instrument Company). and bound radioactivity was counted by a TopCount Microplate Scintillation Counter (Packard Instrument Company).

2.5. Functional studies

The strips (15–20 h after excision) were placed in 5-ml organ baths filled with oxygenated Krebs solution at \(37^\circ\)C and connected to isometric force transducers (Ugo Basile, Varese, Italy) under an initial tension of 20 mN. Mechanical activity was amplified and digitally recorded by an Octal Bridge Amplifier connected to PowerLab/8sp hardware system and analyzed using the Chart 4.2 software (AD Instruments, Australia).
The activity of ibodutant at the human tachykinin NK2 receptors was evaluated against the selective NK2 receptor agonist [bAla^8]NKA(4-10) (Maggi et al. 1993) in the presence of atropine (1μM), indomethacin (3μM) and the selective tachykinin NK1 receptor antagonist SR140333 (0.1μM).

After 60 min stabilization period two reproducible responses to 80 mM KCl were established at 45 min intervals to assess tissue viability. After a further 45 min equilibration period, during which the medium was renewed every 15 min, nifedipine (0.3μM) was added to the Krebs solution and there was left in all subsequent experimental phases to eliminate spontaneous activity (Maggi et al. 1989; Zagorodnyuk et al. 1994). After 30 min of incubation in the presence of nifedipine the preparations were challenged with 1μM [bAla^8]NKA(4-10) to evaluate the contractile response of each preparation.

After a further 90 min equilibration period, concentration-response curves to the tachykinin NK2 receptor selective agonist [bAla^8]NKA(4-10), were cumulatively constructed. In each experiment one strip was pretreated with the vehicle (DMSO: 1–3μl/ml) and used to perform the control curve to [bAla^8]NKA(4-10), while the other strips, obtained from the same specimen, were pre-treated with ibodutant (3, 10, 30 and 100 nM) added to the organ bath 60 min before the concentration-response curve to [bAla^8]NKA(4-10). In each preparation only one cumulative concentration-response curve to [bAla^8]NKA(4-10) was carried out and only one concentration of the antagonist was tested.

In a separate series of experiments the reversibility of tachykinin NK2 receptors blockade produced by ibodutant was evaluated in the human colon preparations using the technique as described by Patacchini et al. (2000) and by Meini et al. (2009). These experiments were also performed in the presence of atropine (1μM), indomethacin (3μM), SR140333 (0.1μM) and nifedipine (0.3μM). After a stabilization period of 60 min, preparations were exposed twice, every 30 min, at a submaximal concentration (171 nM) of [bAla^8]NKA(4-10), calculated from control concentration response curve of the agonist, as the one producing 90% of its maximal contractile effect. After further 30 min, ibodutant (10, 30, 100 and 300 nM) or the vehicle (DMSO, 1–3μl/ml) were added to the bath solution and incubated for 60 min before the next challenge with the agonist (Time 0). After the agonist had produced its maximum contractile effect, the preparations were subjected to a washing protocol of the agonist and the antagonist consisting of three washing periods lasting 10 s each 10 min during which the volume of the organ bath was renewed five times for each washing period (15 renewals in all). Thereafter the administration of the agonist was repeated every 30 min in antagonist-free solution to measure the reversibility of antagonist action for 180 min.

2.6. Data evaluation and statistical analysis

All data in the text or figures are expressed as mean ± standard error of the mean (S.E.M.) or 95% confidence limits (95% c.l.).

Data from radioligand binding experiments were fitted by nonlinear regression using GraphPad Software Prism 4.02 to determine the equilibrium dissociation constant (Kd) from homologous competition experiments performed with NKA, and the ligand concentration inhibiting the radioligand binding of the 50% (IC50) from heterologous competition experiments (ibodutant, saredutant and nepadutant). Kd values were calculated as IC50/bound radioligand. IC50 values were calculated from IC50 using the Cheng-Prusoff equation (Kd = IC50/[1+([radioligand]⁻[bAla^8]NKA(4-10)]) according to the used concentration and the obtained Kd value of the radioligand (Cheng and Prusoff, 1973) in each experimental section (using tissue from the same donor). For graphical presentation data obtained at each concentration of competing ligand, were normalized as percentage of specific binding as follows: [(bound-nonspecific)/specific] × 100.

Functional data were fitted by sigmoidal nonlinear regression (Prism 4.02, GraphPad Software) to determine the agonist concentration producing the 50% (EC50) of the maximal response from the concentration-response curves. Differences in the maximum contractile effects between controls and ibodutant-pretreated preparations were evaluated by one-way Analysis of Variance (ANOVA) and the Dunnett Multiple Comparison Test.

The antagonist potency of ibodutant was expressed in terms of pKb estimated as the mean of the individual values obtained with the Gaddum equation: pKb = log(CR–1) – log[B] were CR is the concentration-ratio calculated from equieffective concentrations of agonist (EC50) obtained in the presence and in the absence of antagonist and B is the used antagonist concentration (Kenakin, 2006).

Competitive antagonism was checked by the Schild regression analysis by plotting the estimates of log(CR–1) against log[B] to determine the slopes of linear regression: a plot with linear regression line and slope not significantly different from unity was considered as proof of competitive antagonism (Arunlakshana and Schild, 1959).

In reversibility experiments of tachykinin NK2 receptor blockade produced by antagonists performed in the human colon preparation responses obtained in each strip at different time were percentaged towards the basal response obtained in each preparation (before antagonist treatment), and compared to those obtained in control time-matched preparations. Data obtained were analyzed by two-way analysis of variance (ANOVA) for repeated measures followed by the Bonferroni post-test.

3. Results

3.1. Radioligand binding inhibition experiments

The characterization of [125I]NKA binding sites was made by means of NKA homologous competitive inhibition curves (Fig. 1). NKA Kd value was 1.77 ± 0.20 nM (n = 7). All tested NK2 receptor antagonists completely inhibited the [125I]NKA specific binding, and the rank order of potency was ibodutant > saredutant > nepadutant. The obtained pKb values were 9.9 ± 0.14 (n = 7) for ibodutant, 9.2 ± 0.06 (n = 7) for saredutant and 8.4 ± 0.15 (n = 7) for nepadutant (Table 1). The analysis of inhibition curves
The affinity \( (pK_a) \) was evaluated in inhibiting the \([\text{[125]}\text{I}]\text{NKA} \) specific binding to membranes prepared from smooth muscle of human colon or from CHO cells stably expressing the human tachykinin NK2 receptor (CHO/hNK2R). The antagonist potency \( (pK_a) \) was evaluated in antagonizing contractile responses induced by \([\text{[Ala}}^8\text{NKA}(4-10)]\) in the human colon smooth muscle or the inositol phosphates accumulation induced by NKA in CHO/hNK2R.

Data are from the present study or from previous measurements performed in our laboratories, as indicated. The affinity \( (pK_a) \) evaluated in inhibiting the \([\text{[125]}\text{I}]\text{NKA} \) specific binding to membranes prepared from smooth muscle of human colon or from CHO cells stably expressing the human tachykinin NK2 receptor (CHO/hNK2R). The antagonist potency \( (pK_a) \) was evaluated in antagonizing contractile responses induced by \([\text{[Ala}}^8\text{NKA}(4-10)]\) in the human colon smooth muscle or the inositol phosphates accumulation induced by NKA in CHO/hNK2R.

* Meini et al., 2009.
* Renzetti et al., 1999.
* Patacchini et al., 2000.

indicated that NKA best fitted according to a one-binding site model, whereas inhibition curves of the three antagonists did not follow the law of mass action, and data were best fitted by a variable slope regression with Hill slope values significantly less than unity: \(-0.70 \text{ (95\% c.l. } -0.80 \text{ to } -0.61) \) for ibodutant, \(-0.82 \text{ (95\% c.l. } -0.93 \text{ to } -0.72) \) for saredutant, and \(-0.63 \text{ (95\% c.l. } -0.71 \text{ to } -0.56) \) for nepadutant.

### Table 1

Comparison of ibodutant and tachykinin NK2 receptor antagonists affinity and potency values detected in native and recombinant bioassays.

<table>
<thead>
<tr>
<th>Binding affinity (( pK_a ))</th>
<th>Ibodutant</th>
<th>Nepadutant</th>
<th>Saredutant</th>
</tr>
</thead>
<tbody>
<tr>
<td>Human colon smooth muscle</td>
<td>9.9</td>
<td>8.4</td>
<td>9.2</td>
</tr>
<tr>
<td>CHO/hNK2R</td>
<td>10.8*</td>
<td>8.5*</td>
<td>9.8*</td>
</tr>
</tbody>
</table>

Antagonist potency \( (pK_a) \)

| Human colon smooth muscle   | 9.1       | 8.3*       | insurm.*   |
| CHO/hNK2R                   | 10.3–10.6*| 8.3*       | 9.8 insurm.*|

insurm. = insurmountable.

### 3.2. Antagonism toward concentration-dependent contractions produced by \([\text{[Ala}}^8\text{NKA}(4-10)]\)

In the presence of atropine (1 \( \mu \)M), indomethacin (3 \( \mu \)M), SR140333 (0.1 \( \mu \)M) and nifedipine (0.3 \( \mu \)M) the selective NK2 receptor agonist \([\text{[Ala}}^8\text{NKA}(4-10)]\) produced slowly developing, concentration-dependent, tonic contractions of the human isolated circular colon: the \( E_{\text{max}} \) was 34.6 \( \pm \) 2.6 mN (n = 13). The \( E_{50} \) value calculated from control (vehicle-treated) concentration-response curves to \([\text{[Ala}}^8\text{NKA}(4-10)]\) was 12.4 nM (95\% c.l. 11.7 – 13.9; n = 13).

Ibodutant (3, 10, 30 and 100 nM) was devoid of any effect on the resting tension of the preparation whereas it concentration-dependently and with high potency antagonized \([\text{[Ala}}^8\text{NKA}(4-10)]\)-induced contractile responses producing a parallel rightward shifts (Fig. 2A) of the agonist response curves without depressing the agonist \( E_{\text{max}} \) (34.6 \( \pm \) 2.6; 33.2 \( \pm \) 2.9; 31.1 \( \pm \) 2.9; 33.3 \( \pm \) 4.9 and 37.0 \( \pm \) 1.7 mN in controls and in the presence of 3, 10, 30 and 100 nM of ibodutant, respectively). Schild plot analysis was consistent with competitive antagonism (slope = 1.02, 95\% c.l. 0.85–1.19) and a \( pK_a \) value of 9.1 \( \pm \) 0.05 was calculated (Fig. 2B).

No gender differences were seen in response to the NK2 receptor-agonist \([\text{[Ala}}^8\text{NKA}(4-10)]\) nor to the antagonist activity of ibodutant. In particular the \( E_{\text{max}} \) to \([\text{[Ala}}^8\text{NKA}(4-10)]\) averaged 30.8 \( \pm \) 3.7 mN and 37.0 \( \pm \) 3.4 mN (n = 5 and n = 8, respectively, n.s.) in strips from male and female, respectively. Likewise the potency of \([\text{[Ala}}^8\text{NKA}(4-10)]\) averaged 15.4 nM (95\% c.l. 12.6–18.9) and 10.3 nM (95\% c.l. 9.3–11.5) (n = 5 and n = 8, respectively, n.s.) in strips from male and female, respectively.

With regard to ibodutant the analysis of its antagonist potency toward \([\text{[Ala}}^8\text{NKA}(4-10)]\) yielded an apparent \( pK_a \) value of 9.0 \( \pm \) 0.1 and 9.1 \( \pm \) 0.05 (n = 18 and n = 31, respectively, n.s.) in colonic strips from male and female patients, respectively.

### 3.3. Reversibility of functional tachykinin NK2 receptor blockade

Reversibility of functional tachykinin NK2 receptor blockade by ibodutant was evaluated by measuring the capability of the human colon muscle to recover the control contractile response produced by a single submaximal concentration (171 nM) of \([\text{[Ala}}^8\text{NKA}(4-10)]\) which induced about 90% of its maximum contractile effect and amounted to 29.7 \( \pm \) 3.2 mN (n = 15).

After the incubation period of 60 min, ibodutant (10, 30, 100 and 300 nM) produced a significant concentration-dependent inhibition of the contractile effect produced by \([\text{[Ala}}^8\text{NKA}(4-10)]\). At this time (Time 0) the inhibitory effect induced by ibodutant was 23 \( \pm \) 9, 55 \( \pm \) 12, 73 \( \pm \) 6 and 84 \( \pm \) 8% at 10, 30, 100 and 300 nM, respectively (Fig. 3). The inhibition remained constant for all concentrations tested with no recovery of the subsequent responses to the agonist obtained in drug-free medium for 180 min.

### 4. Discussion

In this study the pharmacological characterization of the tachykinin NK2 receptor antagonist ibodutant (MEN15596) is presented in the circular smooth muscle of human colon, and the high affinity and antagonist potency, besides the long duration of action of this antagonist, proved also in this tissue.

The determination of ibodutant affinity through radioligand binding experiments indicate that this antagonist recognizes the NKA binding sites present in the human colon with a significant high affinity \( (pK_a, 9.9) \). In the same experiments the affinity of the others tachykinin NK2 receptor antagonists, nepadutant and saredutant, was evaluated as well. Present results indicate that overall the rank order of affinity values for the three antagonists,
i.e. ibodutant (pKi, 9.9) > saredutant (pKi, 9.2) > nepadutant (pKi, 8.4), well matches with that previously obtained at the human recombinant tachykinin NK2 receptor (ibodutant pKᵢ 10.8, saredutant pKᵢ 9.8, nepadutant pKᵢ 8.5; Meini et al., 2009; Renzetti et al., 1999). The affinity of saredutant and nepadutant was previously shown in the human colon also by Warner et al. (1999) and the calculated pKi values are in a similar range (saredutant 9.5 and nepadutant 9.1).

The antagonist potency of ibodutant estimated at the NK2 receptors of the human colon in the present study was similar (pKᵢ 9.1 ± 0.05) to that found in human urinary bladder (pKᵢ = 9.2), guinea-pig colon (pKᵢ = 9.3) and minipig urinary bladder (pKᵢ = 8.8) NK2 receptor smooth muscle preparations (Cialdai et al., 2006), but also in the inositol phosphates accumulation induced by NKA in CHO cells expressing the human NK2 receptor (pKᵢ 10.3–10.6) (Meini et al., 2009).

The Schild analysis yielding to a slope (1.02) not significantly different from unity clearly indicates the competitive antagonist behavior of ibodutant in antagonizing the NK2 receptor-mediated motor responses produced by the application of the selective NK2 receptor agonist [βAla⁶]NKA(4-10). In a previous study Burcher et al. (2008) reported a significantly higher βmax value for NK2 receptor in male as compared to female human colon, although no differences in NK2 receptor mRNA were observed as well as no difference in potency and maximal responses to NKA or [Lys⁴, MeLeu⁸, Nle¹⁰]-NKA(4-10) were detected. Likewise, we failed to detect any gender differences in the contractile response to [βAla⁶]NKA(4-10) nor in the antagonist potency of ibodutant. Overall the results of Burcher et al. (2008) and the present results offer little ground to speculate for a gender-related differential role of NK2 receptors in the genesis of symptoms of IBS.

We have previously shown (Cipriani et al., 2011) that ibodutant prevents internalization of NK2 receptors induced by [βAla⁶]NKA(4-10) in human colon. The receptor internalization is unlikely to be relevant for present findings because: (a) the techniques used to highlight receptor internalization requires a prolonged exposition to the agonist at very low temperature followed by a rapid heating, far different in terms of kinetics from the present experimental conditions; (b) the protocol used in this study enables reproducible responses to NK2 receptor agonists to be performed at relatively short (30 min) time intervals thus indicating that receptors internalization, if any, is negligible.

The kinetic profile of ibodutant was previously observed at molecular level and its fast associating and slow dissociating properties in the interaction with the human tachykinin NK₂ receptor assessed (Meini et al., 2009). In the current investigation, the kinetic of ibodutant interaction at the NK₂ receptor was evaluated by using a functional experimental approach. Present data indicate that ibodutant persistently binds to the human tachykinin NK2 receptor expressed in the human colon smooth muscle, as the inhibition of the motor response induced by [βAla⁶]NKA(4-10) does not recover during the 3h observation period in drug-free medium. These data obtained with ibodutant resemble those previously observed with the tachykinin NK2 receptor antagonist saredutant in the same experimental model (Patacchini et al., 2000). On the other hand, although the functional receptor blockade exerted by ibodutant (present study) or by saredutant (Patacchini et al., 2000) in the present assay appear to be similar, analogs experiments performed in a cell system (Meini et al., 2009) indicated that despite of their slow dissociation, both antagonists exerted a reversible functional blockade. The different behavior obtained in the cell system and in the smooth muscle tissue can be ascribed to the different experimental parameters used, such as the concentrations of used agonist and antagonist and the kinetics of the measured response. On the other hand, despite the very slow dissociation property of both ibodutant and saredutant they are endowed of a different antagonist behavior. In particular although ibodutant slowly reverts from the receptor compartment it exerts a surmountable competitive antagonism type (present study, Meini et al., 2009), whereas saredutant was reported to display an insurmountable antagonism both in contractility smooth muscle (Patacchini et al., 2000) and cell system assays (Meini et al., 2009).

NKA, via NK2 receptors, has been already documented to be a major mediator of the non-adrenergic non-cholinergic (NANC) excitatory input to the circular muscle of human ileum (Maggi et al., 1992) and colon (Cao et al., 2000; Aullı et al., 2008). It appears therefore that the role of NKA as excitatory NANC enteric neurotransmitter, as widely documented to exist in various mammalian species (Holzer and Holzer-Petsche, 1997a,b), is largely maintained in humans and there is abundant evidence associating tachykinins with altered gastrointestinal motility, secretion and visceral sensitivity (Lecci et al., 2004 for review) thus making the field of tachykinins antagonists an appealing target for the development of a new pharmacological treatment of IBS.

The present findings, which document the potent and long lasting NK2 receptor antagonist activity of ibodutant in human colon, support the concept that this molecule is suitable candidate for therapeutic strategies aiming at a control of exaggerated intestinal motility. Ibodutant is currently undergoing Phase II clinical trial in IBS patient with predominant diarrhea.

References


with indomethacin and in diverticular disease and ulcerative colitis.
NK2 receptors mediate neurally induced contraction of human sigmoid
Catalioti, R.M., Crisciouli, M., Cucchi, P., Giachetti, A., Giannotti, D., Giuliani, S.,
Lecci, A., Lippi, A., Patacchini, R., Quartzara, L., Renzetti, A.R., Tramontana, M.,
bicyclic peptide tachykinin NK2 receptor antagonist. Br. J. Pharmacol. 123,
81–91.
Cheng, Y., Prusoff, W.H., 1973. Relationship between the inhibition constant (K1)
and the concentration of inhibitor which causes 50% inhibition (ISO) of an
Cialdai, C., Tramontana, M., Patacchini, R., Lecci, A., Catalani, C., Catalioto, R.M.,
Meini, S., Valenti, C., Altamura, M., Giuliani, S., Maggi, C.A., 2006. MEN15596, a
novel nonpeptide tachykinin NK2 receptor antagonist. Eur. J. Pharmacol. 549,
140–148.
Cipriani, G., Santicioli, P., Evangelista, S., Maggi, C.A., Riccadonna, S., Ringressi,
M.N., Bechi, P., Fausson-Pellegrini, M.S., Vannucchi, M.G., 2011. Effect of
otolinium bromide and ibodutant on the internalization of the NK2 receptor in
mediate non-cholinergic transmission to the circular muscle of the guinea-pig
ization of the binding sites of [3H]SR48968, a potent nonpeptide radioligand
1172–1177.
Giuliani, S., Barbanti, G., Turini, D., Quartzara, L., Rovero, P., Giachetti, A., Maggi, C.A.,
1991. NK2 tachykinin receptors and contraction of circular muscle of the
203, 365–370.
excitation, secretion and inflammation. Pharmacol. Ther. 73, 219–263.
99–126.
receptors in the gut, with special reference to NK2 receptors in human.
Maggi, C.A., Giuliani, S., Patacchini, R., Turini, D., Barbanti, G., Giachetti, A., Meli, A.,
1989. Multiple sources of calcium for contraction of the human urinary
Maggi, C.A., Giuliani, S., Patacchini, R., Santiocioli, P., Theodorrson, E., Barbanti, G.,
contractions in the circular muscle of the human ileum. Gastroenterology 102,
88–96.
Maggi, C.A., Patacchini, R., Rovero, P., Giachetti, A., 1993. Tachykinin receptors and
and NK2 receptor antagonists and atropine-resistant ascending excitatory
reflex to the circular muscle of the guinea-pig ileum. Br. J. Pharmacol. 112,
161–168.
Meini, S., Bellucci, F., Catalani, C., Cucchi, P., Giolitti, A., Santiocioli, P., Giuliani, S.,
2009. Multifaceted approach to determine the antagonist molecular mechan-
ism and interaction of ibodutant ([1-(2-Phenyl-1R-[1-(tetrahydropyran-4-
imethyl)-piperidin-4-ylmethyl]-carbamoyl)-ethylcarbamoyl]-cyclopentyl-]
amide) at the human tachykinin NK2 receptor. J. Pharm. Exp. Ther. 329,
486–495.
nepadutant at tachykinin NK2 receptors in human intestine and urinary
Renzetti, A.R., Catalioti, R.M., Carloni, C., Crisciouli, M., Cucchi, P., Giatoli, A.,
Zappitelli, S., Rotondaro, L., Maggi, C.A., 1999. Effects of tyrosine 289phyla-
lanine mutation on binding and functional properties of the human tachykinin
NK2 receptor stably expressed in chinese hamster ovary cells. Biochem.
Pharmacol. 57, 899–906.
127, 1105–1110.
mediate tachykinin receptor-induced contraction in circular muscle of guinea-