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Activation of the cloned human NK_3 receptor in Chinese Hamster Ovary cells characterized by the cellular acidification response using the Cytosensor microphysiometer

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1 The aim of the present study was to validate the Cytosensor microphysiometer, a novel system that measures the extracellular acidification rate as a reliable index of the integrated functional response to receptor activation, as a method for studying NK_3 receptor pharmacology, and then to use this system to assess the functional activity of novel compounds at this receptor.

2 The selective NK_3 agonist senktide caused reproducible, concentration-related increases in acidification ratein CHO-NK_3 cells, with a pEC_{50} value of 8.72 ± 0.11 ($n=15$). [β-Ala^8]NKA(4–10), the selective NK_2 agonist, elicited a much weaker response ($pEC_{50} = 6.68 \pm 0.08$, $n=4$), while the NK_1-selective agonist substance P methylester only caused a very weak response at concentrations $\geqslant 3$ μM ($n=2$). The rank order of potency for the endogenous tachykinins NKB > NKA > substance P ($n=3$) confirmed the response was mediated by the NK_3 receptor. Moreover, the actual potencies obtained were consistent with affinities measured in radioligand binding studies.

3 The novel compounds PD156319-121 (0.3–1 μM), PD161182 (10–300 nM), PD168001 (10–100 nM) and PD168073 (10–100 nM) all acted as surmountable antagonists of the senktide-induced acidification response, with pA_2 values of 7.49, 8.67, 9.17 and 9.25 respectively ($n=3-5$). In comparison the known NK_3 antagonist SR142801 (10–100 nM) had a pA_2 value of 8.83 ($n=8$) for the interaction with senktide. Again, these values are consistent with the radioligand binding data.

4 Amiloride (1 mM) inhibited the senktide-induced acidification response by 68.3 ± 3.3 ($n=4$), indicating that the Na^+/H^+ antiporter plays an important role in this response, and this is consistent with the importance of this antiporter in other acidification responses.

5 Inhibition of protein kinase C with staurosporine (0.1 μM), or depletion of the intracellular Ca^{2+} stores with thapsigargin (1 μM), both resulted in a reduction in the maximum response to senktide (63.3 ± 1.7 and 68.9 ± 3.2% respectively, $n=3-5$), and co-application of these inhibitors abolished the response ($n=3$). This strongly suggested that the NK_3 receptor was coupling *via* phospholipase C (PLC), as would be expected, although this could not be confirmed by the use of the putative PLC/PLA_2 inhibitor U73122.

6 In conclusion, we have demonstrated the utility of the Cytosensor in the characterization of functional responses to agonists, and assessment of the affinities of antagonists in CHO cells expressing the human NK_3, and have shown that our series of novel compounds are non-peptide NK_3 antagonists of high affinity, as exemplified by PD168073.

Keywords: Tachykinin; Cytosensor; microphysiometry; neurokinin-3 receptor pharmacology; senktide; NK_3 antagonists; signal transduction; PD168073

Introduction

The mammalian tachykinin system consists of three distinct neuropeptides; substance P (SP), neurokinin A (NKA) and neurokinin B (NKB), that preferentially (but not exclusively) act on NK_1, NK_2 and NK_3 receptors respectively (Maggi *et al*., 1993). All are widely distributed throughout the central nervous system and periphery, with putative roles in neuronal signalling, particularly those involved in sensory neurotransmission, and in the regulation of tone in many visceral smooth muscles, and the secretions of exocrine glands (Otsuka & Yoshiota, 1993, Maggi *et al*., 1993).

The NK_3 receptor has been demonstrated functionally in various tissues including the rat portal vein and the myenteric plexus of the guinea-pig ileum (Maggi *et al*., 1993), as well as in brain in the guinea-pig medial habenula (Boden & Woodruff, 1994) and the rat dorsal vagal complex (Carpentier & Baude, 1996). However, studies of the functional role of the NK_3 receptor have been hampered by the lack of high affinity selective antagonists (Maggi *et al*., 1993), although the description of a non-peptide NK_3 antagonist with high affinity has recently been reported (Emonds-Alt *et al*., 1995; Roccon *et al*., 1996).

Shigemoto and colleagues, (1990) cloned and expressed the rat NK_3 receptor, and more recently the human NK_3 receptor has been cloned from brain tissue and subsequently expressed in Chinese Hamster Ovary (CHO) cells (Buell *et al*., 1992). In this system typical NK_3 binding characteristics were observed (Buell *et al*., 1992; Suman-Chauhan *et al*., 1994). Nevertheless, functional studies have revealed discrepancies concerning the signal transduction pathways involved (Nakajima *et al*, 1992; Parsons *et al*., 1995), possibly reflecting the different tissues used. Chung and colleagues, (1994) have demonstrated increased polyphosphoinositide turnover following stimulation of the cloned NK_3 receptor expressed in CHO cells, indicative of phospholipase C (PLC) coupling, and others have confirmed this by showing that senktide mobilizes Ca^{2+} from inositol(1,4,5)trisphosphate ($Ins(1,4,5)P_3$)-sensitive stores in these cells (Pinnock *et al*., 1994). However, there is also

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evidence that cyclic AMP formation is stimulated following NK_3 receptor activation, at least in recombinant systems, although the importance of this second messenger is unclear (Nakajima *et al*., 1992; Parsons *et al*., 1995).

The Cytosensor microphysiometer (Molecular Devices) is a device that quantifies cellular metabolic activity by measuring the changes in the extracellular acidification rate, as a reliable index of the integrated functional response to receptor activation (Owicki *et al*., 1990; McConnell *et al*., 1992; Smart *et al*., 1997). In the current investigation the utility of the Cytosensor for studying NK_3 receptor pharmacology has been confirmed by comparing the activities of known NK_3 agonists and antagonists in the Cytosensor with their affinities in concurrent radioligand binding studies. Furthermore, it has been demonstrated that, as would be predicted, the acidification response reflects the activity of the PLC-coupled signalling pathway. In addition, once validated the Cytosensor was used to assess the functional activity of a series of novel non-peptide compounds, and was used to show that these were high affinity NK_3 antagonists. Part of this work has previously been communicated to the British Pharmacological Society (Jordan *et al*., 1994, 1995).

Methods

Expression of the NK_3 receptor in CHO cells

The human NK_3 receptor was constructed as described previously (Chung *et al*., 1994). Briefly a 0.6 kb fragment containing the C-terminal half of the human NK_3 receptor cDNA was isolated by polymerase chain reaction (PCR) from a human foetal brain cDNA library. The N-terminal portion was synthesized by PCR using overlapping oligonucleotides as templates and their flanking sequences as primers. The two halves were ligated through an overlapping AccI restriction site and the gene sequences confirmed by dideoxy sequencing. The full length gene was then subcloned into the mammalian expression vector pCr/CMV and transfected in to CHO cells.

Cell culture

CHO cells were routinely grown as monolayers in Ham's F12 nutrient media supplemented with 10% foetal calf serum and 2 mM glutamine, and maintained under 5% CO_2 at 37°C. Cells were passaged every 3–4 days, and the highest passage number used was 19. Prior to use in the Cytosensor, the cells were seeded on the polycarbonate microporous membranes of the capsule cups (pore size 3 μm, diameter 12 mm; Molecular Devices) to achieve the density of $\sim$0.6 million cells per cup on the day of the experiment.

Radioligand binding

This was performed as described previously (Suman-Chauhan *et al*., 1994). Briefly, cell membranes were incubated with [I^{125}]-[$MePhe^7$]neurokinin B (40–100 pM) in the presence or absence of test compounds for 90 min at 22°C. Assays were terminated by rapid vacuum filtration. Non-specific binding was defined with 1 μM senktide.

Cellular acidification measurements using the Cytosensor microphysiometer

The Cytosensor microphysiometer (Molecular Devices Corp., California, U.S.A.) has been shown to measure the activity of isolated cells in terms of their rate of production of hydrogen ions. This acidification rate is detected as a change in potential across a silicon light-addressable sensor, during periods of cessation of the flow of medium (McConnell *et al*., 1992). Cells seeded on the special capsule cups (as described above) were placed in sensor chambers at 37°C inside the Cytosensor, and maintained by a flow (120 μl min^{-1}) of non-buffering Ham's F12 media (without $NaHCO_3$). The flow was halted for 22 s at the end of each 2 min pump cycle, and the rate of acidification (μvolts s^{-1}) measured for 15 s during that period. Agonists were introduced sequentially every 30 min to the perfusing media, 20 s before flow-off periods and removed after two rate measurements *via* the automatic valve switch. Where appropriate, various antagonists and signal transduction modifying agents were perfused from 10–30 min prior to the initial challenge, and (with the exception of thapsigargin) then continuously throughout the remainder of the experiment. All agents were applied at the working pH of the medium (=pH 7.4).

Data analysis

Agonists effects were quantitatively determined as the increase in the acidification rate response (peak minus basal), and expressed as a percentage of an initial, standardizing response to 30 nM senktide. Data are presented as means$\pm$s.e.mean unless otherwise stated. Curve fitting and parameter estimation were carried out using GraphpadPrism 1.03. pA_2 values were determined by Schild analysis where the slope was restricted to unity.

Materials

PD156319-121 (Boc(S)Phe(R)αMePheNH$(CH_2)_8CONH_2$), PD161182 ([2-(2,3-Difluorophenyl)-1-methyl-1-(7-ureido-heptylcarbamoyl)-ethyl]-carbamic acid 2-methyl-1-phenyl-propylester), PD168001 ([2-(2,3-Difluorophenyl)-1-methyl-1-(2-morpholin-4-yl-ethylcarbamoyl)-ethyl]-carbamic acid 1-(3-chlorophenyl)-2-methyl-propylester), PD168073 ([2-(2,3-Difluorophenyl)-1-methyl-1-(7-ureido-heptylcarbamoyl)-ethyl]-carbamic acid 1-(3-chlorophenyl)-2-methyl-propylester) were synthesized at Parke-Davis. Thapsigargin was purchased from Calbiochem, Nottingham, U.K. and U73122 from Research Biochemicals Incorporated, Poole, U.K.. Amiloride and stauorsporine were obtained from Sigma, Poole, U.K., while all peptides were supplied by Bachem U.K. All cell culture media were obtained from Life Technologies, Paisley, U.K.. The human foetal brain cDNA was purchased from Clontech, U.K. and plasmid pRc/CMV from Invitrogen, U.K..

Results

Effect of senktide

CHO-NK_3 cells maintained stable resting acidification rates of 0.1–0.35 pH units min^{-1} (100–350 μvolts s^{-1}) within 60 min of the start of perfusion. The NK_3-selective agonist senktide increased the acidification rate, and this response was characterized at higher concentrations by a rapid initial peak followed by a slow recovery to baseline (Figure 1). This response was concentration-related (Figure 1), with threshold around 0.1 nM and maximum at 100–300 nM; the EC_{50} (Table 1) was consistent with activity at the NK_3 receptor. Another NK_3-selective agonist [$MePhe^7$]NKB also produced a concentration-dependent increase in the acidification rate with a

similar potency and E_{max} (Table 1), while the NK_2-preferring agonist [β-Ala^8]NKA(4–10) and NK_1-selective agonist substance P methylester (SPOMe) did elicit responses, but only at markedly higher concentrations (Table 1). The endogenous peptides NKB, NKA and SP also caused similar increases in the acidification rate, with a rank order of potency (Table 1) consistent with activity at the NK_3 receptor. Moreover, these potencies were consistent with the affinities of these agonists in radioligand binding studies (Table 1). In all cases the agonist concentration-response curves had Hill slope values of 0.7–0.8. Inclusion of the peptidase inhibitors phosphoramidon (10 μM), bestatin (10 μM) and captopril (10 μM) had no effect on the acidification response to NKB (EC_{50} of 5.6 nM in the absence, compared to an EC_{50} of 3.8 nM in the presence, of the peptidase inhibitors, $n=4$). Activation of the constitutively expressed P_{2U} receptors with UTP also increased the acidification rate (Table 1).

Antagonism of the senktide acidification response

Perfusion of the novel NK_3 antagonists (Pritchard & Boden, 1995; Suman-Chauhan, unpublished observations) PD161182 (10–300 nM), PD168001 (10–100 nM) or PD168073 (10–100 nM) all resulted in a concentration-related, parallel rightward shift of the senktide concentration-response curve, with no effect on the maximum (e.g. Figure 2), and Schild analysis of these data (e.g. PD168073, Figure 2) yielded slope factors which were not significantly different from unity (Table 2), consistent with competitive antagonism for all the compounds. PD156319-121 also caused a rightward shift of the senktide concentration-response curve, but this compound also inhibited the maximum response, with the Schild analysis yielding a slope factor significantly greater than 1. The pA_2 values for these novel compounds are summarized in Table 2, and compared favourably with the pA_2 of SR142801 (Figure 2, Table 2), a known NK_3 antagonist (Emonds-Alt *et al*., 1995; Beaujouan *et al*., 1997), as well as being consistent with their binding affinities (Table 2). PD168001 and PD168073 also bound to the NK_1 and NK_2 receptors with affinities of 721 (628–822) and 274 (111–573) nM and 1343 (997–1550) and 536 (411–641) nM respectively (range in parentheses, $n=3$ each). SR142801 (10–100 nM) also antagonized the [Me-Phe^7]NKB-induced acidification response, with a pA_2 value of 8.41 ($n=3$).

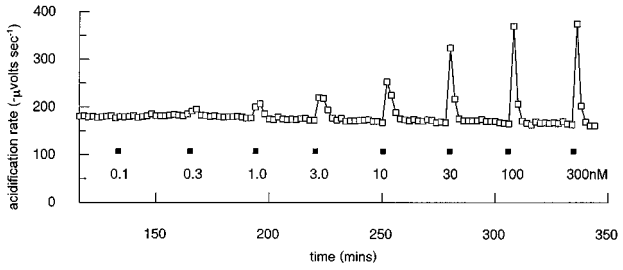

Figure 1 Senktide causes a concentration-dependent increase in the acidification rate in CHO cells expressing the recombinant human NK_3 receptor. The rate of acidification of the media surrounding CHO-NK_3 cells was monitored using a Cytosensor microphysiometer before and during serial perfusion with senktide (0.03–100 nM). Data are a typical trace, representative of $n=15$.

Mechanism of the senktide-elicited acidification response

Inhibition of the Na^+/H^+ antiporter, by the addition of 1 mM amiloride to the perfusion media, caused a substantial reduction in the response to senktide at all concentrations of the agonist between 0.1 and 1000 nM, lowering the maximum response by 68.3±3.3% ($n=4$).

Preincubation of the cells during seeding with cholera toxin (1 μg ml^{-1} for 16 h) or pertussis toxin (200 ng ml^{-1} for 16 h) had no effect on the senktide-induced acidification response (data not shown).

A 10 min perfusion of thapsigargin (1 μM), followed by a 30 min washout, reduced the response to the subsequent addition of senktide throughout the concentration range (68.9±3.2% at E_{max}, $n=5$), while the pEC_{50} (8.64±0.15, $n=5$) was relatively unaltered (Figure 3, upper panel). Blockade of protein kinase C (PKC) with 100 nM staurosporine also reduced the maximum senktide-induced response (63.3±1.7%, $n=3$). A combination of both treatments abolished the senktide-induced response (data not shown, $n=3$). Nevertheless, U73122 (10 μM), the putative PLC inhibitor appeared to have no consistent effect on the maximum senktide-induced response (control=137.8±8.5%, U73122=118.9±22.9%, $n=4$; Figure 3, middle panel), although there were noticeable qualitative changes including early increases in the baseline and some potentiation of the

Table 1 Agonist potency at the recombinant human NK_3 receptor expressed in CHO-K1 cells

Agonist	*pEC_{50}±s.e.mean*	*E_{max}±s.e.mean*	n=	*pK_i* (range)*
Senktide	8.72±0.11	125.8±7.6	15	7.74 (9.00–7.55)
[β-Ala^8]NKA(4–10)	6.68±0.08	117.3±14.1	4	5.79 (6.03–5.60)
SPOMe	—	—	2	>5.00
[MePhe^7]NKB	8.65±0.09	118.4±10.8	3	8.62 (8.96–8.44)
NKB	8.46±0.12	128.6±8.0	3	8.08 (8.87–7.92)
NKA	6.70±0.12	130.8±8.2	3	6.09 (6.37–5.82)
SP	6.36±0.04	126.0±20.8	3	6.21 (6.45–6.04)
UTP	6.08±0.23	155.9±21.6	8	—

*Radioligand binding data from Suman-Chauhan *et al*., 1994, $n=3–5$. All data are for the human receptor, measured using the same ligand as in the present study.

response to lower senktide concentrations (Figure 3, mid- and lower panel). This made reliable interpretation difficult, although in all cases ($n=4$) the senktide concentration-response curve was shifted ~2 fold rightwards in the presence of U73122 (EC_{50} of 14.4 nM in the presence, compared to 8.7 nM in the absence, of U73122; Figure 3).

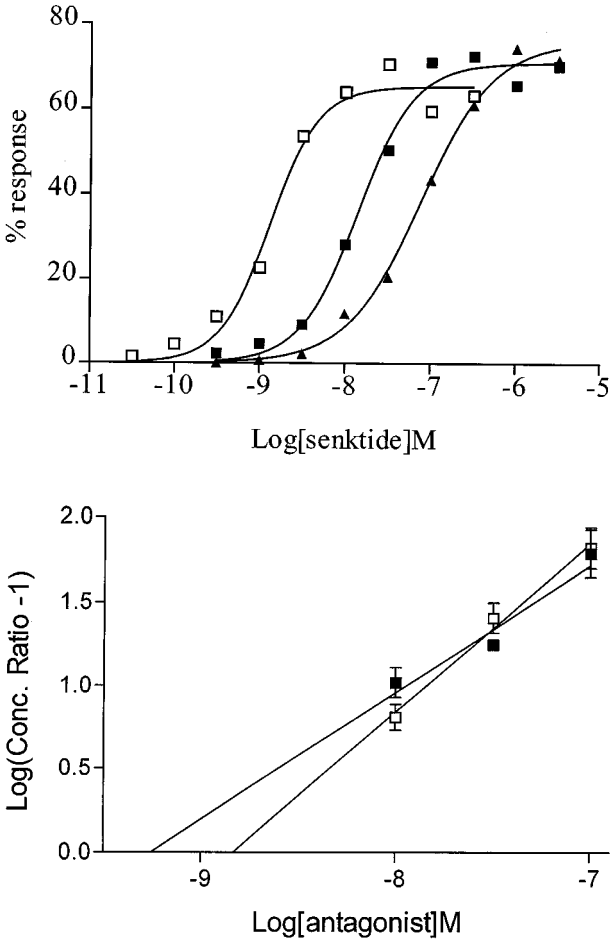

Figure 2 Antagonism of the NK_3 receptor-mediated acidification response by PD168073 and SR142801. Upper panel depicts a typical senktide concentration-response curve (□) in the presence of 10 nM PD168073 (▲) or 10 nM SR142801 (■). Lower panel depicts the Schild analysis of PD168073 (■) and SR142801 (□). The acidification response of CHO-NK_3 cells to senktide (0.03–100 nM), in the presence or absence of PD168073 (10–100 nM) or SR142801 (10–100 nM), was measured using the Cytosensor microphysiometer. Data are means±s.e.mean, where $n=3-8$.

Table 2 Antagonist affinities vs senktide at the recombinant human NK_3 receptor expressed in CHO-K1 cells

Compound	*pA_2*	*slope (95%CL)*	n=	*pIC_{50}#*
PD156319-121	7.49	1.6 (1.05–2.19)	5	7.40*
PD161182	8.67	0.76 (0.44–0.90)	5	8.11*
PD168001	9.17	0.81 (0.58–1.03)	5	8.55
PD168073	9.25	0.77 (0.45–1.08)	3	8.77
SR142801	8.83	1.01 (0.38–1.64)	8	9.69*

#pIC_{50} values are from concurrent radioligand binding studies, and are mean values, where $n=3$. *Data from Pritchard & Boden, 1995. All data are for the human receptor, measured using the same ligand as in the present study.

Discussion

The Cytosensor microphysiometer has been used previously to study the effects of activation of cloned receptors transfected in host cells, and those constitutively expressed in immortal cell lines (Denyer *et al.*, 1993, 1994; Merkouris *et al.*, 1997). In the present study we validated the Cytosensor as a method for studying NK_3 receptor pharmacology, by demonstrating that the potencies/affinities of known NK_3 agonists/antagonists found in this system were comparable with those found in radioligand binding studies conducted in parallel. The Cytosensor was then used for functional

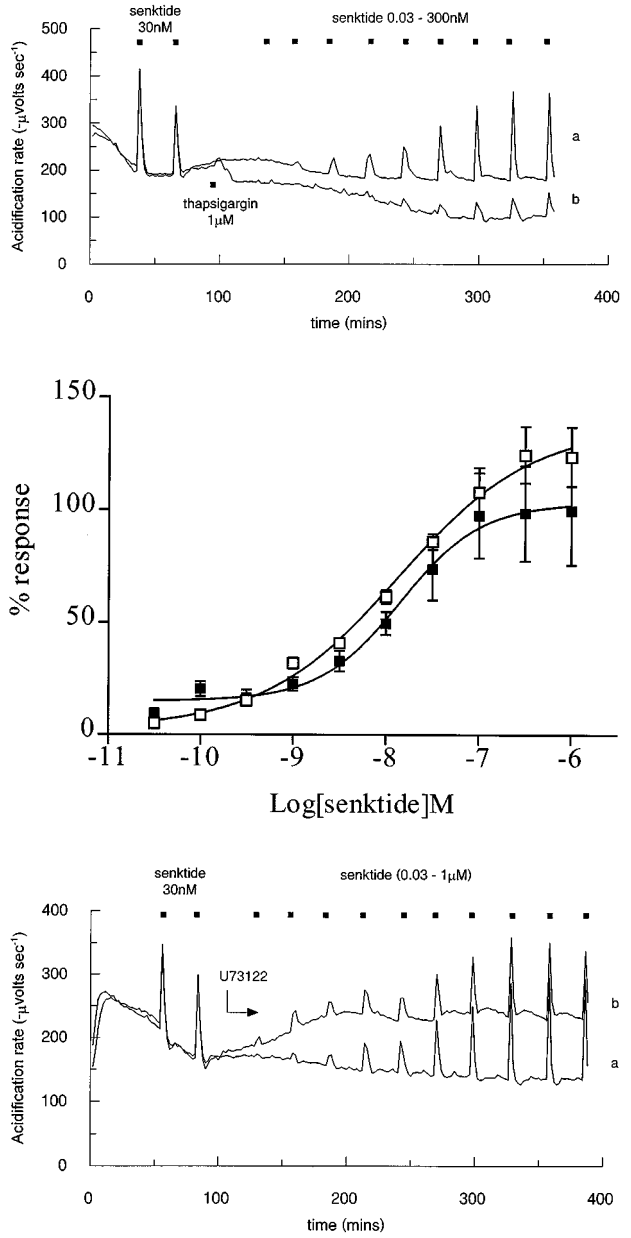

Figure 3 The effects of thapsigargin (upper panel) or U73122 (mid- and lower panels) on the NK_3 receptor-mediated acidification response. The acidification response of CHO-NK_3 cells to senktide (0.03–100 nM), in the presence (b) or absence (a) of thapsigargin (1 µM) or U73122 (10 µM) was monitored using the Cytosensor microphysiometer. Data in the upper and lower panels are typical traces, representative of $n=3$ each, whilst the middle panel depicts a typical senktide concentration-response curve in the presence (■) or absence (□) of 10 µM U73122.

screening of novel compounds, that were shown to be high affinity NK_3 antagonists. Furthermore, as it has been shown previously, using traditional assays, that the NK_3 receptor couples *via* PLC to Ca^{2+} mobilization (Pinnock *et al*, 1994; Parsons *et al*., 1995), we have confirmed that this signalling pathway underlies the acidification response measured in the current investigation.

The NK_3-selective agonist, senktide caused an concentration-related increase in the acidification rate when perfused onto CHO cells expressing the recombinant human NK_3 receptor, indicative of increased cellular activity due to the activation of various signal transduction pathways, as reported for other receptor types (Owicki *et al*, 1990; Smart *et al*., 1997). A variety of other tachykinin agonists also increased the acidification rate, and the rank order of potency for these agents (senktide = [MePhe^7]NKB = NKB > NKA = [β-Ala^8]NKA(4–10) > SP > SPOMe) is consistent with accepted NK_3 receptor pharmacology (Maggi *et al*., 1993). Moreover, both the rank order and actual potencies obtained were also consistent with those from radioligand binding studies. It is worth noting that the agonist concentration-response curves had Hill slope values of 0.7–0.8, suggesting that the agents may have been metabolized. However, the NKB responses were unaffected by peptidase inhibitors, thus indicating the slope factors found may be an artefact of the Cytosensor, possibly due to the response being an integrated function of multiple coupling pathways, each with a slightly different concentration-response relationship. Alternatively, the low slope factors could be representative of some degree of desensitization, although this is unlikely as the basal acidification rate remained relatively constant.

Although the distribution of the NK_3 receptor is well documented (Maggi *et al*., 1993; Otsuka & Yoshiata, 1993), less is known about the functional role of this system, due predominantly to the lack of high affinity, selective antagonists. For example, both GR138676 (Stables *et al*, 1994) and SR48968 (Petitet *et al*, 1993) are NK_3 antagonists with moderate affinity, but have little/no selectivity over NK_1 or NK_2 receptors respectively (Stables *et al*., 1994; Petitet *et al*., 1994). However, in the present study we have demonstrated that the novel compounds PD156319-121, PD161182, PD168001 and PD168073, derived from our earlier chemical leads (Boden *et al*., 1994, Giardina & Raveglia, 1997), are all antagonists of high affinity against the senktide-induced acidification response. In addition, these values are consistent with those obtained from the radioligand binding studies (Pritchard & Boden, 1995; Table 2), that also confirmed that these agents were selective for the NK_3 receptor (Pritchard & Boden, 1995 and results section). Indeed, in the present study PD161182, PD168001 and PD168073 were all competitive antagonists as the slope factors yielded by their Schild analyses were not significantly different from unity. Furthermore, the pA_2 values for these compounds, especially PD168073, compared favourably with the pA_2 value for the NK_3 antagonist SR142801 obtained in the current investigation, as well as with those previously reported for SR142801 using conventional assays (Emonds-Alt *et al*., 1995; Oury-Donat *et al*, 1995; Beaujouan *et al*., 1997) and with the new class of 2-phenyl-4-quinolinecarboxamide derived NK_3 antagonists discovered by SmithKline Beecham (Giardina *et al*., 1996; Giardina & Raveglia, 1997). It is also worth noting that SR142801 is reported to antagonize differentially senktide- and [MePhe^7]NKB-induced responses in the rabbit iris, suggesting that there may be subtypes of the NK_3 receptor (Medhurst *et al*., 1996). Indeed, other studies have also provided evidence for the existence of NK_3 receptor subtypes (Nguyen *et al*., 1994). However, SR142801 produced similar antagonism of both the senktide- and [MePhe^7]NKB-induced acidification responses in CHO cells expressing the human NK_3 receptor in the present study, indicating that receptor subtypes were not present, although the affinity obtained was ~10 fold lower than that identified with binding, probably reflecting the relative sensitivity of the assays.

The NK_3 receptor has been shown to couple to PLC, and so mobilize Ca^{2+} from Ins(1,4,5)P_3-sensitive intracellular stores (Pinnock *et al*., 1994; Parsons *et al*., 1995), although other pathways have also been implicated, at least in recombinant systems (Nakajima *et al*., 1992). Therefore, we examined the mechanisms underlying the NK_3 receptor-mediated acidification response. Firstly, using blockade with 1 mM amiloride, the extent of the contribution of the Na^+/H^+ antiporter in the extrusion of acid from the cells was determined as ~70%, a figure in close agreement with the role of this antiporter in other acidification responses (Denyer *et al*., 1993). Secondly, inhibition of PKC with staurosporine or depletion of the Ins(1,4,5)P_3-sensitive Ca^{2+} stores with thapsigargin were both shown to reduce the senktide-induced acidification response by ~70%, and a combination of these two agents abolished the response, strongly suggesting that both arms of the PLC-linked cascade (Berridge, 1993) are involved in this NK_3 receptor mediated signal transduction process. Similar results were obtained in concurrent Ca^{2+} imaging studies, as previously reported (Pinnock *et al*., 1994). Furthermore, the G-protein involved in the NK_3 receptor mediated acidification response is both pertussis- and cholera toxin-insensitive. However, it is not clear why the putative PLC/phospholipase A_2 inhibitor U73122 failed to inhibit the acidification response, particularly in view of the previously reported profound blockade by U73122 of the senktide-induced increase in intracellular Ca^{2+} response in parallel experiments in the same cells (Pinnock *et al*., 1994). This may represent an artefact caused by non-specific effects of U73122 on the extrusion of acid from the cells because, as described in the results section, there were qualitative changes in the acidification response following the addition of U73122. Alternatively, this lack of inhibition may reflect the contribution of other signal transduction mechanisms to the acidification response, as the NK_3 receptor has been shown to couple to adenylate cyclase as well as PLC, as indeed have the NK_1 and NK_2 receptors, when overexpressed in a recombinant system (Nakajima *et al*., 1992). Nevertheless, taken collectively, the current data provides further evidence that the human NK_3 can couple to PLC, as previously reported (Pinnock *et al*., 1994; Chung *et al*., 1994), and that this mechanism underlies the acidification response measured in the present study.

In conclusion, the Cytosensor microphysiometer has been validated as a technique for assessing the functional effects of activation of the cloned NK_3 receptor expressed in CHO cells, having been shown to produce data consistent both with the findings from concurrent radioligand binding assays and the accepted pharmacology of the NK_3 receptor. Furthermore, the use of the Cytosensor to measure changes in the acidification rate following activation of the NK_3 receptor has been shown to be a reliable method for identifying novel antagonists, as exemplified by PD168073.

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