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Activation of Neurotensin Receptors and Purinoceptors in Human Colonic Adenocarcinoma Cells Detected with the Microphysiometer

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ABSTRACT. Activation of endogenous neurotensin (NT) receptors and P_2-purinoceptors expressed by human colonic adenocarcinoma HT-29 cells increased extracellular acidification rates that were detected in the microphysiometer. NT (pGlu-Leu-Tyr-Glu-Asn-Lys-Pro-Arg-Arg-Pro-Tyr-Ile-Leu), NT[8–13] (Arg-Arg-Pro-Tyr-Ile-Leu), NT[9–13] (Arg-Pro-Tyr-Ile-Leu), and NT1 (N^{α}methyl-Arg-Lys-Pro-Trp-Tle-Leu [Tle = *tert*-leucine]) were full agonists, whereas XL 775 (*N*-[*N*-[2-[3-[[6-amino-1-oxo-2-[[(phenylmethoxy)carbonyl]-amino]hexyl]amino]phenyl]-3-(4-hydroxyphenyl)-1-oxo-2-propenyl]-L-isoleucyl]-L-leucine) was a partial agonist for activating NT receptors expressed by HT-29 cells. Desensitization induced by NT was rapid and monophasic with 85% of the initial response lost by a 30-s exposure. Once initiated, the rate and extent of desensitization were similar for different concentrations of a given agonist, for agonists of different potencies, and for agonists of different efficacies, which suggests that desensitization may be independent of receptor occupancy or agonist efficacy. Resensitization was a much slower process, requiring 60 min before the full agonist response to NT was recovered. ATP, via P_2-purinoceptors, also activated cellular acidification rates in a concentration-dependent manner. ATP induced a biphasic desensitization of purinoceptors with a loss of ca. 50% of the initial stimulation detectable between 30 and 90 s of exposure to the agonist. Desensitization of NT receptors did not influence the activation of P_2-purinoceptors by ATP, suggesting there was no heterologous desensitization between the two types of receptors. Superfusion with NT receptor agonists for 15 min at concentrations that did not elicit changes in extracellular acidification rates blocked, in a concentration-dependent manner, the agonist response induced by 100 nM NT. This may reflect sequestration of the receptor. These results suggest that the high agonist affinity state of NT receptors may modulate receptor sequestration, whereas activation of the low agonist affinity state may be linked to cellular metabolism. Comparison of our results with published data found differences as well as similarities of NT responses among three lines of HT-29 cells. BIOCHEM PHARMACOL **54**;7:825–832, 1997.

KEY WORDS. HT-29 cells; NT receptor; P_2-purinoceptor; microphysiometer

Human colonic adenocarcinoma (HT-29) cells express endogenous P_{2U}-purinoceptors [1] and neurotensin (NT)‖ receptors. The latter comprise a single class of high affinity binding sites for NT [2–6], which are insensitive to levocabastine [4, 6]. Agonist activation of both types of receptors increases the turnover of phosphatidylinositols [2, 3, 7] and transiently increases intracellular calcium [3]. Unlike cell lines and tissues derived from the nervous system, which express high affinity NT receptors that increase cyclic nucleotide turnover [8, 9], no changes in cyclic AMP or cyclic GMP were induced by NT in HT-29 cells [2].

The NT receptor cloned from HT-29 cells has an amino acid sequence 84% identical [4] to rat brain NT receptors [10]. Although there is a high degree of correlation among agonists binding to NT receptors in epithelia-derived human HT-29 cells and rat CNS, pharmacological data suggest that there may be differences in NT receptors in these tissues [6].

Characteristic of endogenously expressed NT receptors is a rapid desensitization of functional responses with little or no change in the number of cell surface receptors. This was clearly demonstrated in HT-29 cells by Turner *et al.* [3]. In addition, this group showed heterologous desensitization between NT receptors and other receptors coupled to PI turnover in HT-29 cells including ATP-activated receptors [3].

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‖ *Abbreviations:* DMEM, Dulbecco's modified Eagle's medium; NT, pGlu-Leu-Tyr-Glu-Asn-Lys-Pro-Arg-Arg-Pro-Tyr-Ile-Leu; NT[8–13], Arg-Arg-Pro-Tyr-Ile-Leu; NT[9–13], Arg-Pro-Tyr-Ile-Leu; NT1 (N^{α}methyl-Arg-Lys-Pro-Trp-Tle-Leu [Tle = *tert*-leucine]; PI, phosphatidylinositol; XL 775, *N*-[*N*-[2-[3-[[6-amino-1-oxo-2-[[(phenylmethoxy)carbonyl]amino]-hexyl]amino]phenyl]-3-(4-hydroxyphenyl)-1-oxo-2-propenyl]-L-isoleucyl]-L-leucine.

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The microphysiometer, a silicon-based biosensor, detects changes in the metabolic activity of cultured cells by monitoring the rate of extracellular acidification [11]. This rate depends on metabolic activity, a parameter dependent on cell type [11]. In the microphysiometer, a voltage signal proportional to pH is detected every second; a signal of 1 μV/s corresponds to changes of approximately 1×10^{-3} pH units per min [12]. Accumulation of extracellular acidic metabolites is monitored by periodically stopping the flow of medium across the cells for a short period (seconds). The signal elicited by modulating activity of receptors, ion channels, enzymatic processes, etc. [13] is subtracted from the basal rate and expressed as a percent of this basal rate.

As a means to detect activation of G protein-coupled receptors, the microphysiometer displays greater sensitivity compared with measuring second messenger formation. For example, we could detect no change in IP_1 formation in our line of HT-29 cells activated by 100 nM NT, and only a small increase was observed with 100 μM ATP, whereas these agonists elicited reproducible signals in the microphysiometer (see "Results"). Greater sensitivity in the microphysiometer has also been shown for muscarinic receptors expressed at different receptor densities [14]. Other advantages of the microphysiometer include measurements of cellular responses in real time, the absence of radioactivity, and a less labor-intensive technique than radiolabeled second messenger formation. In addition, the microphysiometer requires a smaller number of cells and greatly facilitates repeated measurements on the same cell population compared with determinations of intracellular calcium fluxes in cell suspensions. The greater ease of handling data from the microphysiometer compared with fluorescence signals of calcium fluxes in individual cells allows a greater throughput for compound testing. In addition, the microphysiometer is not restricted to detecting responses of G protein-coupled receptors or receptor-coupled ion channels and may also monitor cellular responses elicited by growth hormones, insulin, cytotoxic compounds, enzyme activators or inhibitors, and other biologically active agents [12, 15].

The objective of this study was to evaluate the microphysiometer as a detection system to screen compounds for functional responses at NT receptors. In addition, we compared responses of NT receptors in HT-29 cells using the microphysiometer with measurements of intracellular calcium mobilization reported in the literature [3, 6] as well as determining potential interactions between ATP-activated and NT receptors in these cells. Although there seemed to be differences among the three lines of HT-29 cells (see "Discussion"), the strong correlation in the pharmacological responses observed in these studies supports the utility and advantages of the microphysiometer as a tool for monitoring receptor activation.

MATERIALS AND METHODS

Cell Culture

HT-29 cells were kindly provided by Dr. P. Kitabgi (Université de Nice-Sophia Antipolis, Valbonne, France). The cells were maintained in culture in Dulbecco's modified Eagle's medium (Gibco, Life Technologies European Division, Eragny, France) containing 1% L-glutamine, 10% heat-inactivated fetal calf serum, 1 mM sodium pyruvate, 1% Pen-Strep (Gibco), and 8% CO_2. Cells were renewed after approximately 22 passages. For experiments with the microphysiometer, HT-29 cells were seeded into capsule cups (Molecular Devices Ltd., Crawley, UK) 72 or 96 hr before use at a density sufficient to obtain ca. 3×10^5 cells/cup on the day of the experiment.

Binding

Saturation experiments were performed to determine receptor density and the dissociation constant (K_D) for NT. HT-29 cell membranes (ca. 30 μg of protein) were incubated in 50 mM TRIS-HCl (pH 7.4) containing 1 mg/mL bovine serum albumin, 1 mM $MgCl_2$, 1 mM phenanthroline, 1 μM thiorphan, 40 μg/mL bacitracin, 4 μg/mL leupeptin, and ^{125}I-NT in a final volume of 0.5 mL. Incubation (3 hr at room temperature) was terminated by rapid filtration. Specific binding was measured in the presence of 1 μM NT. Protein content was determined using the Bradford method [16].

Microphysiometer Parameters

The capsule cups containing HT-29 cells were set up in the Cytosensor™ (Molecular Devices Ltd. UK) prewarmed to 37° and superfused with "running medium" made with serum-free, bicarbonate-free DMEM (Gibco), to which was added 2.1 g/L NaCl and 1% Pen-Strep. The pump speed was 50% of maximum (100 μL/min). The pump cycle time was 60 s, which included a 20-s pump-off, during which changes in the cell acidification rate were measured for 15 s.

Pharmacological Parameters

After at least 1 h of superfusion to obtain stable responses, all chambers were superfused with 100 nM NT, which served as the control stimulation (S1) for each superfusion chamber. Subsequent stimulations with agonists or with NT in the presence of the antagonist SR 48692 were calculated as ratios of S1 (i.e. Sx/S1). Unless otherwise indicated, cells were stimulated with NT or other agonists added from inlet 2 of the microphysiometer for 10 s before the pump-off period (a 10-s stimulation). Responses to agonists were sharp peaks, which were quantified by reading from the screen the peak height above basal. To block agonist responses, cells were superfused with different concentrations of test compound for 15 min before stimulation with NT.

TABLE 1. Intrinsic activity (R_{max}) and potency (EC_{50}) of agonists, blockade (IC_{50}) of NT receptor activation in HT-29 cells, and the ratios of EC_{50}/IC_{50} values

	Agonist Activity			Blockade of Agonist Response to NT		
Agonist	n	EC_{50} (nM)	R_{max}	n	IC_{50} (nM)	EC_{50}/IC_{50}
NT[8–13]	6	10 ± 1	1.13 ± 0.04	3	0.32 ± 0.04	31
NT	13	20 ± 3	1.08 ± 0.04	3	0.95 ± 0.24	21
NT1	8	131 ± 30	1.02 ± 0.09	4	22 ± 4	6
NT[9–13]	4	265 ± 31	0.99 ± 0.07	4	26 ± 1	10
XL-775	3	14000 ± 1900	0.27 ± 0.07*	3	10400 ± 2700	1

Values are means ± SEM for (n) experiments.
*, $p < 0.05$ compared with all other agonists.

Calculations

Concentration-response curves (response calculated as the ratio of a given stimulation to the control stimulation, Sx/S1) were fitted to a logistic function using GraFit (Erithacus Software, Ltd., London, UK) or SigmaPlot (Jandel Scientific, Erkrath, Germany). Parameters calculated included maximum responses (R_{max}), the concentration of an agonist that induced half the maximal response for that agonist (EC_{50}), and the concentration that blocked the maximal response to NT by 50% (IC_{50}). The EC_{50} and R_{max} values listed in Table 1 are means calculated from individual experiments except that for XL-775, which lists the EC_{50} value calculated from the averaged fitted curve. Statistical differences were calculated using single-factor ANOVA and Scheffe's F test.

Drugs and Chemicals

All cell culture materials and the running medium were purchased from Gibco Life Technologies (SARL, Eragny, France). NT was purchased from Sigma Chimie (St. Quentin, France). NT[8–13] and NT[9–13] were synthesized under contract with Prof. Dr. G. Jung (University of Tübingen, Germany). Purity was reconfirmed in house; there were no detectable levels of NT[8–13] in the sample of NT[9–13]. NT1 was synthesized by Neosytem Laboratoire (Strasbourg, France). XL-775 and SR 48692 were synthesized in house by A. Zimmermann and B. Lesur, respectively.

RESULTS

Pharmacology of the Neurotensin Receptor in HT-29 Cells

The HT-29 cells used in this study had a neurotensin receptor density of 18 ± 2 fmol of binding sites for [^{125}I]NT per mg cell membrane protein; the K_D for the radioligand was 0.07 ± 0.01 nM (n = 4 from two separate experiments).

The basal acidification rate of HT-29 cells before the initial stimulation was 302 ± 9 μV/s (calculated from experiments on 28 days; the average basal rate of 1 day's experiment was considered as n = 1); the range was from 198 to 400 μV/s. These values were set equal to 100%, and S1 was calculated as percent above basal. The average 10-s stimulation with 100 nM NT increased the acidification rate by 117 ± 6% above basal (n = 28), i.e. slightly more than double the basal response.

NT, NT[8–13], NT[9–13], NT1, and XL-775 activated cellular metabolism in a concentration-dependent manner (Fig. 1). With the exception of XL-775, the compounds induced similar maximal responses (Table 1)and were thus considered to be full agonists. The intrinsic activity of XL-775 was significantly less than that of the other agents and was classified as a partial agonist. The nonpeptide antagonist SR 48692 alone had no effect on cell acidification rates (data not shown) but concentration-dependently blocked the responses induced by 100 nM NT (Fig. 2). The values for this antagonist calculated from the concentration-response curves were IC_{50} = 240 ± 40 nM and slope = 1.22 ± 0.16 (n = 5).

Desensitization of Neurotensin Receptors Induced by Agonists and Resensitization of the Response to NT

Extracellular acidification rates in the presence of 20 and 100 nM NT, 100 nM NT[8–13], 0.1 and 1 μM NT1, and 100 μM XL-775 decreased with increasing times of expo-

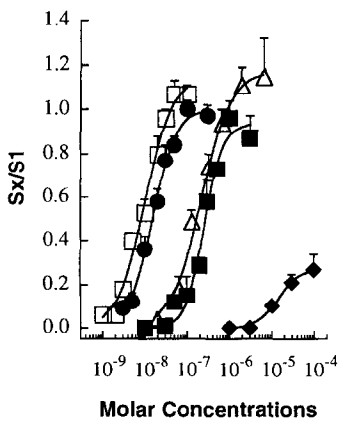

FIG. 1. Agonist concentration-response curves at neurotensin receptors on HT-29 cells. NT[8–13] (□, n = 6), NT (●, n = 13), NT1 (△, n = 8), NT[9–13] (■, n = 4), and XL-775 (◆, n = 3). Values are means ± SEM for n experiments.

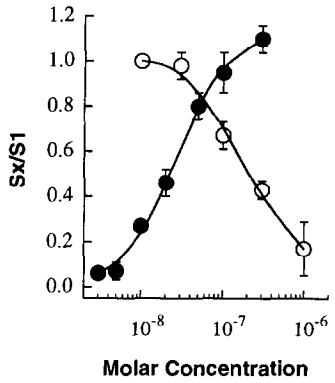

FIG. 2. Concentration-dependent stimulation by NT (S2, ●) and concentration-dependent inhibition by SR 48692 (added to superfusion medium 15 min before S3) of cellular acidification rates elicited by 100 nM NT (S3, ○). Values are means ± SEM of five experiments.

sure (Fig. 3, A and B, x axis in seconds). In the continuing presence of 100 nM NT or 100 nM NT[8–13], there was a monophasic decline in the cellular response such that, after a 30-s superfusion, responses to these agonists were ca. 15% of S1. NT at 20 nM stimulated extracellular acidification at the same rate for 10 and 15 s, and the response then

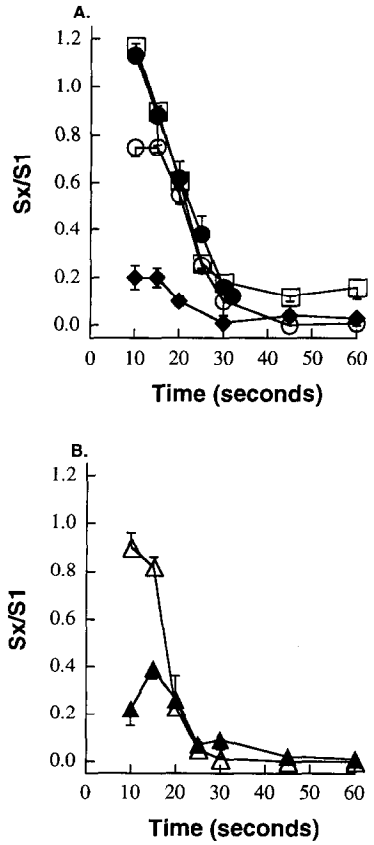

FIG. 3. Response with increasing time of exposure (seconds of superfusion before measuring cellular response). A, 100 nM NT (●, n = 5), 20 nM NT (○, n = 3), 100 nM NT[8–13] (□, n = 5), and 100 μM XL-775 (◆, n = 3). B, 0.1 μM NT1 (▲, n = 4), and 1 μM NT1 (△, n = 5). Values are means ± SEM for n experiments.

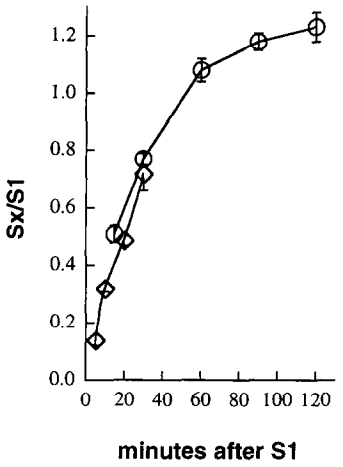

FIG. 4. Recovery of the response to a 10-s stimulation of 100 nM NT. After the initial stimulation (S1), cells were retested for the response to 10 s of 100 nM NT at 15, 30, 60, 90, and 120 min after S1 (○, n = 6) or 5, 10, 20, and 30 min after S1 (◇, n = 4). Values are mean ratios of Sx/S1 ± SEM for n experiments.

decreased at the same rate as observed with 100 nM NT. NT1 was a full agonist of lower potency than NT[8–13] or NT (Table 1), but the rate and extent of desensitization were similar to that of the more potent agonists. The response to 0.1 μM NT1 was greater at 15-s superfusion than at a 10-s superfusion, and the response then decayed at a similar rate and to a similar extent as that induced by 1 μM NT1 (Fig. 3B). In the presence of a concentration of XL-775 that produced a maximal response (Fig. 1), the acidification rates, equal to 20% of S1, were similar at 10 and 15 s of superfusion. The response then declined to less than 5% of S1 at 30 to 90 s of superfusion (Fig. 3A).

Resensitization was determined by measuring the cellular response to a second 10-s stimulation by 100 nM NT at intervals of 20 to 120 min after S1 (Fig. 4, x axis in minutes). In another series of cells, resensitization was tested at times up to 30 min after S1; the two values at 30 min were in close agreement (Fig. 4). The HT-29 cells remained desensitized for at least 5 min after a 10-s stimulation with 100 nM NT and then began to slowly recover. At least 60 min were required before the amplitude of the initial stimulation was restored. Unless otherwise indicated, stimulations were spaced 90 min apart.

Other Phosphatidylinositol-linked Receptors in HT-29 Cells

In the microphysiometer, a challenge for 10 s with ATP added 5 min after 100 nM NT for 30 s (i.e. neurotensin receptors desensitized, see Fig. 3A) produced a concentration-dependent response (Fig. 5A) with the maximum similar to that obtained with ATP in the absence of NT (Fig. 5B). The EC_{50} for ATP was 16 ± 5 μM, and the slope was 1.04 ± 0.10 (n = 4 for both values). Desensitization was also observed with ATP, but the kinetics differed from those seen with NT receptor agonists. The responses at a

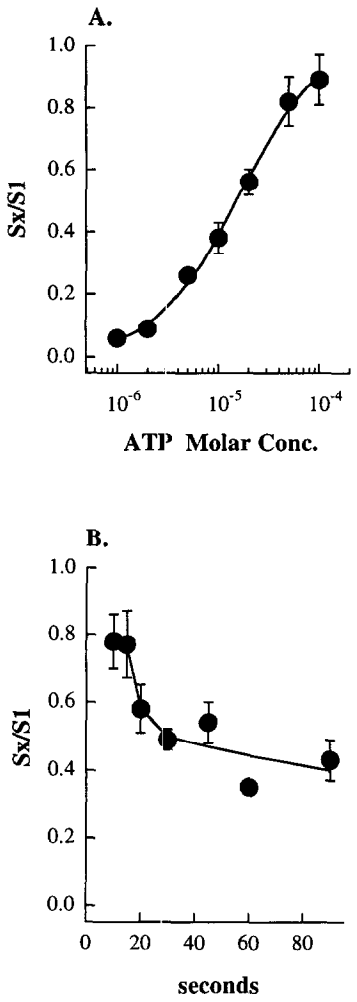

FIG. 5. A, response to increasing concentrations of ATP (10-s stimulation) 5 min after superfusion with 100 nM NT for 30 s. The responses to ATP were calculated as ratios of the control response to NT (S1). Values are means ± SEM for $n = 4$. B, response to 100 μM ATP with increasing time of exposure (seconds of superfusion before measuring cellular response). Values are means ± SEM for $n = 4$.

10-s and a 15-s exposure to 100 μM ATP were similar, indicating that, for the P_2-purinoceptors endogenously expressed in HT-29 cells, the maximum tissue response was observed. At exposure times greater than 15 s, there was a biphasic decrease in the response to 100 μM ATP, a rapid phase followed by a slower decrease between 30 and 90 s to ca. 40% of the initial response. In addition to NT, carbachol and ATP have also been reported to increase intracellular calcium mobilization in HT-29 cells [3]. However, in our line of HT-29 cells, 1 mM carbachol, superfused for 10 to 60 s failed to modify basal cellular acidification rates (data not shown). NT stimulated IP3 accumulation in HT-29 cells [3]. The effect of 100 nM NT or 100 μM ATP on IP1 formation was determined in HT-29 cells using a previously published protocol [17]. In two separate experiments (results not shown), no response was observed with NT; ATP produced a small (<2 times basal) stimulation.

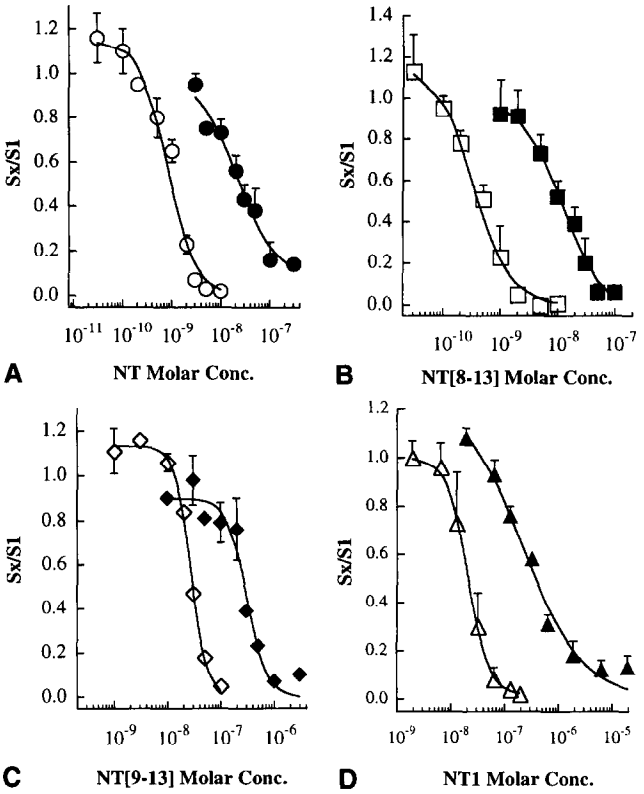

FIG. 6. Tachyphylaxis (filled symbols) or desensitization (hollow symbols) at neurotensin receptors in HT-29 cells. Tachyphylaxis was induced by a 10-s stimulation with increasing concentrations of test compound; 5 min later the cells were restimulated with 100 nM NT (10 s). Desensitization (hollow symbols) was induced by increasing concentrations of test compound superfused for 15 min before stimulation with 100 nM NT (10 s). Values are means ± SEM. A, NT (●, $n = 4$; ○, $n = 3$). B, NT[8–13] (■, $n = 3$; □, $n = 3$). C, NT[9–13] (◆, $n = 3$; ◇, $n = 4$). D, NT1 (▲, $n = 3$; △, $n = 4$).

Tachyphylaxis of Neurotensin Receptors Induced by NT, NT[8–13], NT[9–13], and NT1

HT-29 cells were superfused for 10 s with different concentrations of NT, NT[8–13], NT[9–13], and NT1. Five minutes later, the cells were challenged for 10 s with 100 nM NT. All four agonists induced a concentration-dependent decrease in the second stimulation with NT (Fig. 6, A–D, solid symbols). The initial 10-s stimulation with 100 and 300 nM NT decreased the response to the second stimulation with NT (14 ± 6% and 13 ± 3% of S1, respectively, $n = 4$) to a similar extent as with 100 nM NT for 30 s (16 ± 1%, $n = 4$).

Blockage of the Neurotensin Agonist Response

Superfusion for 10 s with low concentrations of NT, NT[8–13], NT1, and NT[9–13] elicited no detectable changes in extracellular acidification rates. However, superfusion for 15 min at these concentrations diminished the response to a subsequent challenge for 10 s with 100 nM NT in a concentration-dependent manner (Fig. 6, A–D, hollow symbols). For example, superfusion for 10 s with 5

nM NT barely elicited an agonist response (12 ± 4% of S1 (n = 13), Fig. 1), whereas this concentration superfused for 15 min depressed the response to the challenge stimulation with 100 nM NT by 96 ± 2% (n = 3). Table 1 lists the IC_{50} values calculated from the curves of a concentration-dependent blockade to an agonist challenge with 100 nM NT.

DISCUSSION

In this communication, we present data obtained from activation of endogenous neurotensin receptors and P_2-purinoceptors expressed by HT-29 cells as measured in the microphysiometer. In these cells, both NT and ATP concentration-dependently increased the extracellular acidification rate.

The potency of NT in activating NT receptors (Table 1) was similar to that reported for calcium mobilization in HT-29 cells (EC_{50} = 7 nM, ref. [3]; EC_{50} = 12 nM, ref. [6]. The close agreement is somewhat surprising given the differences in the NT receptor densities (fmols of NT binding sites/mg protein) of 18 (this study), 130 [6], and 484 [3]. The responses elicited by NT in the microphysiometer may have such a large degree of amplification between receptor activation and extracellular acidification that the relatively low receptor density was not a limiting factor for this response.

The rank order of agonist potencies to activate NT receptors in HT-29 cells for NT, NT[8–13], NT1, and NT[9–13] was identical for the microphysiometer (Table 1) and intracellular calcium mobilization [6]. All four compounds were full agonists in both systems, and the EC_{50} values for NT, NT[8–13], and NT1 differed by less than threefold (Table 1; ref. [6]). In contrast, NT[9–13] was eight times more potent in the microphysiometer (EC_{50} = 265 nM) than in mobilizing intracellular calcium (EC_{50} = 2 μM, ref. [6]). The IC_{50} values for the nonpeptide antagonist SR 48692 obtained in these two functional assays in HT-29 cells differed by less than two-fold (see "Results"; ref. [18]). These similarities between published agonist and antagonist potencies and agonist rank orders with the values obtained using the microphysiometer support the validity of the latter for studying agonist-receptor interactions. However, differences in the ratios of some agonist potencies may indicate differences in receptor-effector coupling efficiencies between the two lines of HT-29 cells.

In the continuing presence of either NT or ATP, acidification rates decreased (Figs. 3 and 5), suggesting desensitization of the two types of receptors. In contrast to the rapid, monophasic desensitization induced by 100 nM NT (Fig. 3), desensitization induced by 100 μM ATP was slower in onset and was biphasic, with a plateau from 30 to 90 s of 40–50% of the initial response (Fig. 5B).

Desensitization of NT receptors did not change the response to ATP (Fig. 5A), indicating a lack of heterologous desensitization in our line of HT-29 cells in contrast to the reported heterologous desensitization of NT receptors induced by preincubation with ATP or carbachol in HT-29 cells [3].

Functional responses of NT receptors desensitize more rapidly than changes in binding sites. In HT-29 cells, cellular acidification rates were depressed by 85% after a 30-s exposure to NT (Fig. 3), and intracellular calcium mobilization was maximally depressed (ca. 75%) 1 min after the application of the agonist, whereas NT binding sites were unaffected [3]. There are potential phosphorylation sites on intracellular loops of the NT receptor [10] that may play a role in receptor-effector uncoupling without changing the number of cell-surface receptors. Although protein kinase C activation uncouples NT receptors in HT-29 cells, this does not seem to play a role in agonist-induced desensitization [3].

Preexposure to different concentrations of NT inhibited, in a concentration-dependent manner, a second stimulus by NT added 3 [3] or 5 min later (Fig. 6, A–D). Tachyphylaxis to NT in HT-29 cells is clearly due to desensitization/sequestration of NT receptors and can be detected for up to 60 min after the initial stimulus (Fig. 3).

The rapid desensitization of NT receptors in HT-29 cells observed in the microphysiometer suggests that the maximal tissue response to NT receptor activation by high concentrations of potent agonists may not be detected before desensitization begins to attenuate the response. The similar magnitude of responses at 10 and 15 s induced by 20 μM NT (EC_{50}), by the less potent agonist NT1 at 1 μM, or by the partial agonist XL-775 suggests that under these conditions a full tissue response was attained. In contrast, the responses elicited by a 10- and 15-s exposure to 0.1 μM NT1 (close to the EC_{50} value, see Table 1) may reflect the interplay between equilibration and desensitization. The kinetics of desensitization of NT receptors were similar for full agonists of differing potencies (with NT1 ca. 13 times less potent than NT[8–13]) and for agonists of different efficacies (partial compared with full agonists); the rate and extent of desensitization were also independent of agonist concentration (Fig. 3). These data suggest that, once initiated, receptor-G protein uncoupling continues independent of receptor occupancy or agonist efficacy.

At longer exposure times to agonists (5 mins or more), a decrease in the number of NT binding sites [3] as well as diminished functional responses have been observed in HT-29 cells. In these cells, there was a full recovery of the number of cell surface NT receptors within 20 min after removal of NT, whereas calcium mobilization was still inhibited 60 min later [3]. This contrasts with our results, where a full recovery of the functional response was observed 60 min after removal of the agonist (Fig. 4). The reason for this difference is not known; differences in assay conditions or differences in the cells may be contributing factors.

Superfusion for 15 min with concentrations of agonists that did not elicit changes in cellular acidification rates blocked subsequent NT activation of cellular metabolism. XL-775 antagonized receptor activation by NT with an IC_{50} value similar to its EC_{50} value as would be expected from a partial agonist blocking the response to a full agonist. On the other hand, the apparent antagonism

induced by low concentrations of the four full agonists may be due to receptor sequestration. Internalization by intact cells of radiolabeled NT at subnanomolar concentrations [19, 20] indicates that internalization can occur at low receptor occupancy via a high agonist affinity state (receptors coupled to G proteins). For many G protein-coupled receptors, receptor internalization was dependent on G proteins but independent of second messenger formation, receptor phosphorylation, and desensitization [21–25]. Our results are consistent with agonist activation of NT receptors coupled to G proteins (high agonist affinity state), which does not elicit a cellular response, i.e. no change in second messengers and downstream cellular metabolism, but does lead to receptor sequestration.

That NT receptors may exist in at least two agonist-affinity states is supported by recent binding data using the nonpeptide antagonist [^{3}H]SR48692 as radioligand in guinea pig brain [26] and LTK cells transfected with rat brain NT receptors [27]. In Table 1, the agonist concentrations that half-maximally increased the cellular acidification rate (EC_{50}) were compared with the concentrations that half-maximally inhibited (IC_{50}) the agonist response to 100 nM NT; these ratios decreased with decreasing agonist potency. It has been observed with muscarinic (and other G protein-coupled receptors) agonists that the greater the agonist intrinsic efficacy, the greater the difference in the affinities for the two agonist affinity states of the receptor [28]. Smaller ratios were observed with the lower potency full agonists NT1 and NT[9–13]. The partial agonist XL 775 did not seem to differentiate agonist affinity states of NT receptors expressed in HT-29 cells.

In conclusion, using the microphysiometer, we have detected activation and desensitization of two endogenous receptors expressed by HT-29 cells and compared the NT responses with published reports. The similarity of the pharmacology, desensitization, and resensitization of NT receptors in HT-29 cells determined by measurements of intracellular calcium or the microphysiometer supports the utility of the latter as a tool for measuring receptor activation. There were also differences detected among the three studies using HT-29 cells. There were significant differences in NT receptor densities, responses to a low-potency agonist (NT[9–13]), lack of heterologous desensitization between purinoceptors and NT receptors, and the kinetics of NT resensitization. The use of cultured cells in the drug-discovery process is a fundamental technique, but the above observations underscore the precautions that should be applied when generalizing from data obtained with cultured cells, both among cells from the same line as well as other types of cells or tissues.

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