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DESENSITIZATION OF NEUROTENSIN RECEPTOR-MEDIATED CYCLIC GMP FORMATION IN NEUROBLASTOMA CLONE N1E-115

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Abstract—Murine neuroblastoma clone N1E-115 possesses receptors that specifically bind the tridecapeptide neurotensin, mediate the formation of intracellular cyclic GMP, and stimulate inositol phospholipid hydrolysis. These cells also rapidly degrade neurotensin in a sequential fashion. We studied the effect of prolonged exposure of cells to neurotensin on subsequent neurotensin receptor-mediated intracellular cyclic GMP formation under conditions that prevented degradation of this peptide [J. A. Gilbert and E. Richelson, *Soc. Neurosci. Abstr.* **12**, 762 (1986)]. Neurotensin receptor-mediated cyclic GMP formation in neuroblastoma clone N1E-115 was decreased following prolonged exposure of intact cells to nondegraded neurotensin. The time course of this desensitization was very rapid; the maximal effect on cyclic GMP production (reduction to 10–30% of control values) occurred within 5 min of exposure of intact cells to neurotensin. This desensitization was homologous, as cells desensitized by neurotensin demonstrated no decrease in their cyclic GMP response to angiotensin II (1 μM) or bradykinin (10 nM). Neurotensin preincubation with intact N1E-115 cells for increasing lengths of time caused time-dependent shifts to the right of the dose–response curve and reductions in the maximum cyclic GMP response. Desensitization was reversible, but resensitization was a slower process than desensitization: full recovery of cyclic GMP production required incubation of the desensitized cells for at least 10 min at 37°. From binding studies with [^{3}H]neurotensin, we found that both the apparent equilibrium dissociation constant, K_D, and the maximum number of receptor sites, B_{max}, for this radioligand were decreased significantly ($P < 0.05$) for completely desensitized cells from those values for control cells. These data suggest that desensitization of the neurotensin receptor involved an uncoupling of the pathway of events connecting receptor activation to intracellular cyclic GMP formation; complete desensitization involved both the apparent loss of neurotensin receptors on the cellular surface and the increase in affinity of the remaining receptors for the agonist. This decrease in B_{max} is more likely to be a result of intracellular sequestration of recyclable NT receptors than of true down-regulation due to the rapid resensitization seen for the NT-mediated biological response.

Desensitization is defined as the loss of responsiveness of a tissue to an agonist following lengthy exposure to that agonist. Specific or homologous desensitization is the temporary loss of a single biological response in a multiply-sensitive preparation following extended activation at a specific receptor; non-specific or heterologous desensitization affects multiple receptors mediating the same or different responses and is considered to result from an event induced at some point beyond the individual receptors but before the measured biological responses (see review by Triggle [1]). Desensitization is generally rapidly induced and readily reversible, whereas the loss of response in a cellular system due to loss of receptors or down-regulation may occur rapidly but is more slowly reversible.

We have been studying neurotensin (NT§) receptor desensitization using a neuroblastoma clone as a model for the intact neuron. Murine neuroblastoma clone N1E-115 possesses a single class of receptors which specifically bind the tridecapeptide neurotensin and mediate the formation of intracellular cyclic GMP and the stimulation of inositol phospholipid hydrolysis [2, 3]. Studies with N1E-115 cells that have been cultured in medium without antibiotics and supplemented with 10% fetal bovine serum indicate that radiolabeled neurotensin binds to receptors on intact cells with an apparent equilibrium dissociation constant, K_D, of 11 nM; the concentrations of neurotensin inducing 50% of the maximal response, EC_{50}, in stimulating intracellular cyclic GMP production and inducing inositol phospholipid hydrolysis are 1.5 nM and 0.9 nM respectively [4, 3]. Intact N1E-115 cells also rapidly degrade neurotensin in a sequential fashion [5, 6]. To date, studies employing clone N1E–115 are supportive of neurotensin's putative neurotransmitter status; that is, functional receptors for neurotensin are found on neuronal-like cells, and a mechanism exists for the rapid inactivation of this peptide near the receptor site.

Degradation of neurotensin (10 nM) by clone N1E-115 (1×10^5 intact cells/ml) is inhibited almost

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§ Abbreviations: NT, neurotensin; K_D apparent equilibrium dissociation constant; and B_{max}, maximum number of receptor sites.

completely at 37° by a combination of two protease inhibitors [5, 6]: 1,10-phenanthroline (1 mM) and Z-Pro-Prolinal (1 μM). In experiments analyzing by high pressure liquid chromatography the degradation of [^{3}H]neurotensin by N1E-115 cells, we found that 7 ± 1% (mean ± SEM) of the neurotensin was degraded under these conditions after 20 min compared to 95 ± 1% of [^{3}H]neurotensin incubated identically except for the absence of the two protease inhibitors [*vide supra*]. Therefore, the experiments reported here characterizing the desensitization of the functional neurotensin receptors on neuroblastoma clone N1E-115 following lengthy exposure to neurotensin utilized the described conditions of incubation employing 1,10-phenanthroline and Z-Pro-Prolinal. These data have been presented previously [7].

MATERIALS AND METHODS

Cell culture. Murine neuroblastoma cells (clone N1E-115) were cultured in Dulbecco-Vogt's modification of Eagle's medium (Grand Island Biological Co., Grand Island, NY) without antibiotics and supplemented with 10% (v/v) fetal bovine serum (Grand Island Biological Co.). Cells (passage number <21) were cultured in 20 ml of medium in 75 cm^2 Corning flasks (Corning Glass Works, Corning, NY) in a humidified atmosphere of 10% CO_2:90% air at 37°. Flasks were routinely inoculated with 0.5 to 1.0×10^6 cells that were fed daily beginning day 6 after subculture by removal of 10 ml of growth medium and replacement with 10 ml of fresh medium. Cells were harvested during the stationary phase of growth, 10–22 days after subculture.

Measurement of relative changes in cyclic GMP production. The assay used for measurement of intracellular cyclic GMP formation was essentially that of Richelson *et al.* [8], in which cyclic [^{3}H]GMP produced in clone N1E-115 was isolated chromatographically from cells labeled with radioactive precursor ([^{3}H]guanosine) prior to receptor stimulation. Radioactivity was measured in an Isocap/300 liquid scintillation counter (Searle, Des Plaines, IL).

Neurotensin receptor binding assay. Competition studies measuring the inhibition in binding of [^{3}H]neurotensin to intact N1E-115 cells by increasing concentrations of unlabeled neurotensin were performed essentially by the method of Gilbert *et al.* [4] as follows. N1E-115 cells were harvested for use by aspiration of culture medium, incubation of the cellular monolayer for 10 min at 37° in 10 ml of modified Puck's D_1 solution without antibiotics and phenol red [9], disruption of the layer by agitation of the flask, and collection of the cells by centrifugation at 300 *g* for 1 min at 4° in a CRU-5000 centrifuge (Damon/IEC Division, Needham Heights, MA). The cellular pellet was resuspended in 10–20 ml of phosphate-buffered saline solution (solution I) consisting of 110 mM NaCl, 5.3 mM KCl, 1.8 mM $CaCl_2$, 1.0 mM $MgCl_2$, 2.0 mM Na_2HPO_4, 25 mM glucose and 70 mM sucrose (pH 7.35; 340 milliosmolal), and the washed cells were collected by centrifugation. The cellular pellet was resuspended in at least 2 ml of solution I, and 20 μl of the suspension was removed for enumeration of cells (Coulter Electronics, Hialeah, FL). Prior to distribution to assay tubes, the cellular solution was diluted in solution I to provide 4×10^5 cells per assay tube and was maintained at 0° before use.

[^{3}H]Neurotensin binding to N1E-115 cells was performed at 0° in solution I made 0.1% (w/v) in bovine serum albumin. A total assay volume of 1 ml was used in 5-ml polypropylene tubes (Walter Sarstedt, Inc.), and the reaction was initiated by the addition of cells. During the incubation at 0°, all assay tubes were mixed every 10 min to prevent settling of the cells. Each concentration of competitor was tested in triplicate. Non-specific binding was determined with a 1 μM concentration of unlabeled neurotensin in reaction tubes, and typically was 60% of the total binding. The inhibition experiments were designed with one set each of total and non-specific tubes and with a radiolabel concentration of 3–4 nM. The assay was routinely terminated after 20 min by simultaneous filtration of twenty-four samples on a cell harvester filtering unit (Brandel, Gaithersburg, MD) equipped with a GF/B filter strip (Whatman, Clifton, NJ) which had been pretreated with 0.1% polyethylenimine for 30–60 min just before use. Each tube and corresponding filter were immediately rinsed with three 4-ml rinses of cold solution I. The filters were then placed in plastic scintillation vials (Research Products International Corp., Mt. Prospect, IL) to which 7 ml of Safety-Solve (Research Products International Corp.) counting solution was added, and the filters were allowed to incubate for at least 4 hr prior to radioactivity measurement.

The data were analyzed with the LIGAND program [10] on a 9845B desktop computer (Hewlett–Packard, Fort Collins, CO).

Exposure of intact N1E-115 cells to neurotensin for prolonged periods. To study the effect of prolonged exposure of N1E-115 cells to neurotensin on subsequent neurotensin receptor-mediated intracellular cyclic GMP formation, incubation conditions were employed in which neurotensin degradation is prevented [5, 6]. Thus, cells were harvested, enumerated and prelabeled with [^{3}H]guanosine. This suspension was then diluted in solution I to provide 1×10^5 cells/ml for incubation with nondegraded neurotensin. Aliquots of the cellular solution were distributed into large beakers on the platform of a 37° water bath at 80 oscillations/min (GCA/Precision Scientific, Chicago, IL). The cells were equilibrated for 1 min with the protease inhibitors 1,10-phenanthroline (1 mM) and Z-Pro-Prolinal (1 μM) prior to the addition of solution I (for control cells) or a final concentration of 10 nM neurotensin (for desensitized cells). After an appropriate length of time, the incubation was stopped by placing a beaker on ice and immediately diluting the contents with a volume of ice-cold solution I which was 0.7 times the volume of the experimental sample. The cells from each sample were then pelleted by centrifugation at 300 *g* for 30 sec at 4° and were washed free of any residual NT and protease inhibitors by resuspension in 0.7 vol. of ice-cold solution I. The cells were again collected by centrifugation, and the pellet was suspended in ice-cold solution I to provide 1×10^5 cells/270 μl. This suspension was kept on ice

until the cells were distributed into Linbro multiwell plates (Cat. No. 76-033-05, Flow Laboratories, McLean, VA) for assay at 37°. After warming the cells for 1 min, the cyclic GMP assay described earlier was completed.

To study the effect of prolonged exposure of clone N1E-115 to neurotensin on subsequent neurotensin receptor binding, the described incubation conditions were employed for exposing intact cells to nondegraded peptide. After the cells were harvested and enumerated, they were diluted in solution I to provide 1×10^5 cells/ml for incubation with nondegraded NT. The cells were distributed into two large beakers at 37°, equilibrated for 1 min with the protease inhibitors, 1,10-phenanthroline (1 mM) and Z-Pro-Prolinal (1 μM), exposed to solution I or 10 nM neurotensin for 5 min, and washed free of residual NT and protease inhibitors as described earlier in this section. After the final centrifugation, the collected cells were suspended in ice-cold solution I to provide 4×10^5 cells per assay tube. This suspension was kept on ice until the cells were distributed into tubes for assay at 0°. The NT receptor binding assay was completed as described earlier.

Materials. Polypropylene or polyethylene plasticware were used for all experiments. [^{3}H]Guanosine was from ICN Radiochemicals (Irvine, CA), [^{3}H]neurotensin was obtained from New England Nuclear (Boston, MA), and cyclic [^{14}C]GMP was purchased from Amersham (Arlington Heights, IL). Sigmacote, bovine serum albumin (A-7906), 1,10-phenanthroline, and polyethylenimine were supplied by the Sigma Chemical Co. (St. Louis, MO). Neurotensin was supplied by Boehringer Mannheim (Indianapolis, IN). Z-Pro-Prolinal was donated by Dr. Sherwin Wilk, the Mount Sinai Medical Center, New York, NY. All other reagents were analytical grade.

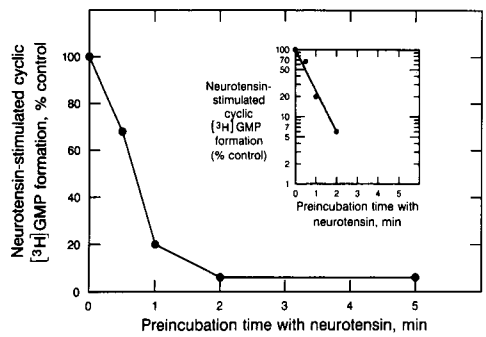

Fig. 1. Time course for desensitization of neurotensin receptor-mediated cyclic GMP formation in clone N1E-115. N1E-115 cells (passage number 18; 17 days after subculture) were harvested and prelabeled with [^{3}H]guanosine as described in Materials and Methods. After dilution to 1×10^5 cells/ml in solution I, the suspension was distributed into separate beakers, and each was equilibrated for 1 min at 37° and 80 oscillations/min with 1,10-phenanthroline (1 mM) and Z-Pro-Prolinal (1 μM) prior to the addition of solution I (for control cells) or 10 nM neurotensin (for desensitized cells). After an appropriate length of time, the incubation was stopped by placing a beaker on ice and adding 0.7 vol. of ice-cold solution I. The cells from each sample were pelleted by centrifugation at 300 *g* for 30 sec at 4° and were washed free of any residual NT and protease inhibitors by resuspension in 0.7 vol. of ice-cold solution I followed by centrifugation. Each cellular pellet was suspended in ice-cold solution I to provide 100,000 cells/270 μl and was kept on ice until used. To assay each sample, the cells were distributed into a Linbro multiwell plate at 37°, warmed for 1 min, and stimulated with solution I (to obtain basal cyclic [^{3}H]GMP values) or 0.1 μM neurotensin; the cyclic GMP measurement was completed as described in Materials and Methods. Each time point was performed with six replicates; the number plotted was obtained by subtracting the average of the basal values from the average of the NT-stimulation data; this figure was then compared to the calculated value obtained with the control cells. The calculated 100% value represents 0.6×10^4 dpm/10^6 cells. *Inset* represents a semilogarithmic plot of the given cyclic GMP data fitted with the least squares method of linear regression analysis. The data presented are from one of three independent experiments.

RESULTS

Under conditions that prevented neurotensin degradation, preincubation of neuroblastoma clone N1E-115 with 10 nM neurotensin markedly and rapidly reduced receptor-mediated cyclic GMP formation in cells subsequently stimulated with 0.1 μM neurotensin (Fig. 1). Neurotensin-induced cyclic GMP production decreased to its lowest levels, 10–30% of control values, following exposure of intact cells to nondegraded neurotensin for less than 5 min. As indicated in the inset of Fig. 1, the rate of desensitization appeared to be first-order, with a half-time of 1.5 ± 0.5 min (mean ± SEM; number of independent experiments = 3).

Desensitization of neurotensin receptor-mediated cyclic GMP formation in clone N1E-115 was reversible, but resensitization (Fig. 2) was a slower process than desensitization. Thus, recovery of full NT-induced cyclic GMP production, 90–100% of control levels, was observed following incubation of the desensitized cells at 37° in the absence of neurotensin for a minimum of 10 min before subsequent neurotensin-stimulation (number of independent experiments = 3).

Neurotensin-induced desensitization was homologous or specific: cells desensitized by exposure to neurotensin demonstrated no decrease in their cyclic GMP production upon subsequent stimulation by agonists specific for other peptide receptors on these cells, that is, angiotensin II (1 μM) [11] or bradykinin (10 nM) [12] (Fig. 3). These neurotensin-desensitized cells, however, were refractory to subsequent neurotensin (0.1 μM) activation.

Dose–response curves for neurotensin-stimulated cyclic GMP production were determined for control cells and for cells incubated with agonist for 2 or 5 min (Fig. 4). Neurotensin preincubation with intact N1E-115 cells for increasing lengths of time caused time-dependent shifts to the right of the dose–response curve and reductions in the maximum cyclic GMP response.

Finally, to determine if the decrease in neurotensin receptor-mediated cyclic GMP formation following desensitization by NT was accompanied by a decrease in the number of neurotensin receptors on N1E-115 cells and a change in the affinity of these

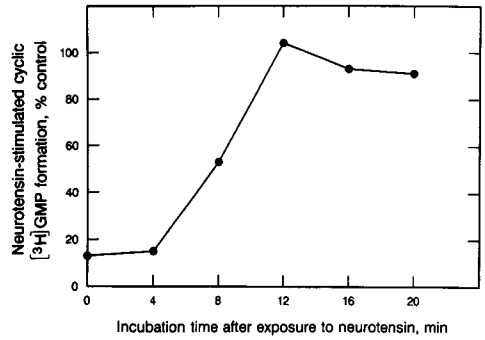

Fig. 2. Time course for reversal of desensitized neurotensin receptor-mediated cyclic GMP formation in clone N1E-115. N1E-115 cells (passage number 11; 14 days after subculture) were harvested and prelabeled with [^{3}H]guanosine as described in Materials and Methods. After dilution to 1×10^5 cells/ml in solution I, the suspension was distributed into two beakers, and each was equilibrated for 1 min at 37° and 80 oscillations/min with 1,10-phenanthroline (1 mM) and Z-Pro-Prolinal (1 μM) prior to the addition of solution I (for control cells) or 10 nM neurotensin (for desensitized cells). After 5 min, the incubation was stopped by placing each beaker on ice and adding 0.7 vol. of ice-cold solution I. The cells from each sample were pelleted by centrifugation at 300 g for 30 sec at 4° and were washed free of any residual NT and protease inhibitors by resuspension in 0.7 vol. of ice-cold solution I followed by centrifugation. Each cellular pellet was suspended in ice-cold solution I to provide 100,000 cells/270 μl and was kept on ice until used. To assay each sample, the cells were distributed into a Linbro multiwell plate at 37°, warmed for an appropriate length of time, and stimulated with solution I (to obtain basal cyclic [^{3}H]GMP values) or 0.1 μM neurotensin; the cyclic GMP measurement was completed as described in Materials and Methods. Each time point was performed in triplicate; the number plotted was obtained by subtracting the average of the basal values from the average of the NT-stimulation data obtained using NT-desensitized cells; this figure was then compared to the value similarly calculated from data provided by control cells which had been incubated in Linbro plates for the same length of time. The calculated 100% value represents 1.9×10^4 dpm/10^6 cells. The data presented are from one of three independent experiments.

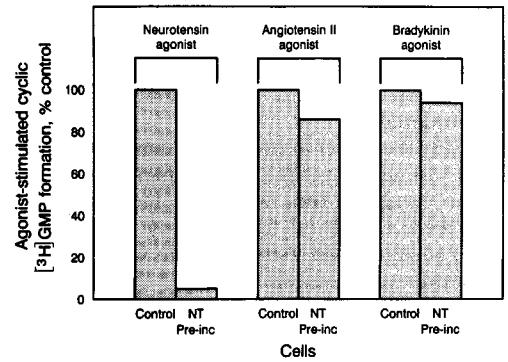

Fig. 3. Specificity of neurotensin desensitization. Neuroblastoma N1E-115 cells (passage number 9; 20 days after subculture) were preincubated for 5 min in the presence of 1,10-phenanthroline (1 mM) and Z-Pro-Prolinal (1 μM) with either solution I (for control cells) or 10 nM NT (for desensitized cells), washed, warmed for 1 min in Linbro multiwell plates, and assayed in triplicate for agonist-induced cyclic [^{3}H]GMP formation during a 30-sec stimulation period. Details of the experimental and computational procedures are given in the legend to Fig. 1. The calculated 100% value represents 1.0×10^4 dpm/10^6 cells. The concentrations of neurotensin, angiotensin II, and bradykinin used as agonist are, respectively, 0.1 μM, 1 μM, and 10 nM. The data presented are from one of three independent experiments.

receptors for the agonist, the specific binding of [^{3}H]neurotensin to control and completely desensitized cells was measured. Table 1 demonstrates that both the apparent equilibrium dissociation constant, K_D, and the maximum number of receptor sites, B_{max}, were significantly smaller for neurotensin receptors on completely desensitized cells compared to those on control cells. Upon complete desensitization, neurotensin receptors on N1E-115 cells had a 3-fold higher affinity for neurotensin and were 3-fold fewer in number.

DISCUSSION

For years this laboratory has been studying murine neuroblastoma clone N1E-115 as a model for the intact neuron. Several types of receptors have been identified on these cells, and some of these receptors, upon activation by specific agonist, stimulate the formation of intracellular cyclic GMP, a compound generally considered the second messenger of some neurotransmitters in the mammalian nervous system [13]. These receptors include the histamine H_1[14], muscarinic (M_1) acetylcholine [8], neurotensin [2],

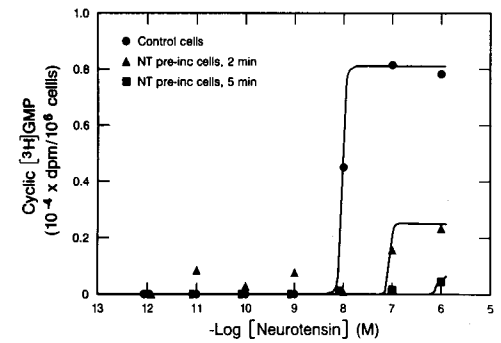

Fig. 4. Effect of preincubation of clone N1E-115 with neurotensin on subsequent neurotensin-induced dose–response curves of cyclic GMP formation. Neuroblastoma N1E-115 cells (passage number 12; 14 days after subculture) were preincubated in the presence of 1,10-phenanthroline (1 mM) and Z-Pro-Prolinal (1 μM) for 5 min with solution I (for control cells) or for 2 or 5 min with 10 nM neurotensin (for desensitized cells), washed, warmed for 1 min in Linbro multiwell plates, and stimulated for cyclic [^{3}H]GMP formation with increasing concentrations of neurotensin. Details of the experimental procedure are given in the legend to Fig. 1. Each point is the average of triplicates from which has been subtracted the average of triplicate basal values obtained using identically preincubated cells. The data presented are from one of three independent experiments.

Table 1. [^{3}H]Neurotensin binding to intact N1E-115 cells

Cells	K_D (nM)	B_{max} (sites/cell)
Control cells	9 ± 2	71,000 ± 19,000
NT-preincubated cells, 5 min	3.3 ± 0.7*	25,000 ± 5,000*

Values are means ± SEM; number of independent experiments = 6.

* Significant difference ($P < 0.05$) from control cells using a two-tailed *t*-test for two means.

bradykinin [12], angiotensin II [11] and thrombin [15] receptors. Previous studies have demonstrated that receptor-stimulated intracellular cyclic GMP formation mediated by the histamine H_1 and the muscarinic acetylcholine receptors becomes desensitized upon lengthy exposure of the N1E-115 cells to specific agonist [16, 17]. Prior to the studies presented here, however, it was not known whether any of the numerous peptide receptors on clone N1E-115 were regulated by desensitization.

Initial attempts to measure NT-induced desensitization of the cyclic GMP response were delayed by the discovery that clone N1E-115 rapidly degrades this peptide into inactive fragments in a sequential fashion [5, 6]. [^{3}H]Neurotensin (0.1 μM) incubated in solution I with 1×10^6 cells/ml at 37° is degraded to an average of 3% of original levels after 5 min of exposure to intact cells [6]. Under these conditions, neurotensin receptors on N1E-115 cells exhibited only partial desensitization to neurotensin [*vide supra*]. Further studies, however, provided incubation conditions under which neurotensin degradation could be essentially prevented at 37° for 20 min, a period long enough to enable the measurement of any desensitization that might occur. Thus, to study the effect of prolonged exposure of cells to neurotensin on subsequent neurotensin receptor-mediated intracellular cyclic GMP formation [7], conditions were employed in which neurotensin degradation is prevented: the incubation of 10 nM neurotensin with 1×10^5 intact cells/ml in a phosphate-buffered saline solution at 37° with inclusion of the protease inhibitors 1,10-phenanthroline (1 mM) and Z-Pro-Prolinal (1 μM).

Like desensitization of intracellular cyclic GMP production in N1E-115 cells mediated by the histamine H_1 and the muscarinic receptors [16, 17], desensitization of neurotensin receptor-induced cyclic GMP formation was rapid, first-order, reversible, and homologous. Furthermore, as seen with muscarinic-receptor-induced desensitization, neurotensin preincubation with intact N1E-115 cells for increasing lengths of time caused both time-dependent shifts to the right of the dose–response curve and reductions in the maximum cyclic GMP response. One possible interpretation of these results is that the pathway of events connecting neurotensin receptor binding to guanylate cyclase activation becomes uncoupled during the development of the desensitized state, as increasing quantities of neurotensin no longer stimulated the same level of cyclic GMP production induced in control cells. The fact that NT-induced desensitization was specific suggests that this uncoupling might involve the physical separation of the NT receptor from its effector or a modification of the receptor protein itself preventing its subsequent interaction with pathway components necessary for cyclic GMP formation.

Using an indirect assay for agonist binding, that is, competition between a radioligand antagonist, pirenzepine, and carbachol, Cioffi and El-Fakahany [18] recently presented evidence that the rapid desensitization of muscarinic M_1 receptors on clone N1E-115 cells is associated with a selective loss of these binding sites that have low affinity for agonist. The remaining binding sites for carbachol bound this agonist with lower affinity. Because neurotensin is an agonist with high affinity for its receptor, we were able to measure directly the binding of this peptide to its receptor site. Our results show that, like the muscarinic M_1 receptor, complete desensitization of neurotensin receptor-mediated cyclic GMP formation in clone N1E-115 was accompanied by changes in both the neurotensin receptor number and the affinity of the neurotensin receptor for its agonist. Binding of [^{3}H]neurotensin to intact N1E-115 cells was measured at the point at which a complete loss of biological response occurred following lengthy neurotensin exposure (5 min). It was found that the number of neurotensin receptors had decreased to one-third of the original number, and the affinity of the receptors for neurotensin had increased 3-fold.

Thus, complete NT-induced desensitization was accompanied by the loss of NT receptors on the cellular surface and the increase in the affinity of the remaining receptors for the NT ligand. This decrease in B_{max} cannot be considered true down-regulation due to the rapid resensitization seen for the NT-mediated biological response (recovery of full cyclic GMP production was seen after 10 min). The loss of NT receptors seen with complete desensitization is more likely to be a result of intracellular sequestration of recyclable NT receptors. As a comparison, the mechanism of desensitization of β-adrenergic receptor-mediated cyclic AMP formation has been well characterized (see review by Sibley and Lefkowitz [19]). In general, homologous desensitization of this receptor involves several steps, the first of which is the rapid decrease in β-adrenergic receptor-induced adenylate cyclase activity coincident with the loss of high-affinity receptors on the cellular surface. It is thought that the nondegraded receptors are sequestered into a membrane or intracellular compartment and are thereby separated from the other components of the adenylate cyclase system. This step is reversible with resensitization of adenylate cyclase activity occurring concomitantly with the reappearance of receptors on the cellular surface. A slower, second step is manifested as down-regulation, with the disappearance of β-adrenergic receptors detected by the lack of binding of radioligand to any subcellular fraction.

Just as the loss of neurotensin receptors on the cellular surface seen with complete desensitization has some resemblance to another receptor desensitization system, so does the increase in affinity for ligand seen with the remaining NT receptors in the

refractory state. As was described in 1957 in the classic model of Katz and Thesleff [20], the desensitized state of the nicotinic acetylcholine receptor has a higher affinity for cholinergic agonists by 1–2 orders of magnitude than does the original, resting form of the receptor (see review of Barrantes [21]).

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