The p75NTR-induced Apoptotic Program Develops through a Ceramide-Caspase Pathway Negatively Regulated by Nitric Oxide*

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SK-N-BE neuroblastoma cell clones transfected with p75NTR and lacking Trk neurotrophin receptors, previously reported to undergo extensive spontaneous apoptosis and to be protected by nerve growth factor (NGF) (Bunone, G., Mariotti, A., Compagni, A., Morandi, E., and Della Valle, G. (1997) Oncogene 14, 1463–1470), are shown to exhibit (i) increased levels of the pro-apoptotic lipid metabolite ceramide and (ii) high activity of caspases, the proteases of the cell death cascade. In the p75NTR-expressing cells, these parameters were partially normalized by prolonged NGF treatment, which, in addition, decreased apoptosis, similar to caspase blockers. Conversely, exogenous ceramide increased caspase activity and apoptosis in both wild-type and p75NTR-expressing cells. A new p75NTR-expressing clone characterized by low spontaneous apoptosis exhibited high endogenous ceramide and low caspase levels. A marked difference between the apoptotic and resistant clones concerned the very low and high activities of nitric oxide (NO) synthase, respectively. Protection from apoptosis by NO was confirmed by results with the NO donor S-nitrosoacetylpenicillamine and the NO-trapping agent hemoglobin. We conclude that the p75NTR receptor, while free of NGF, triggers a cascade leading to apoptosis; the cascade includes generation of ceramide and increased caspase activity; and the protective role of NO occurs at step(s) in between the latter events.

Apoptosis (programmed cell death) is now recognized to play fundamental roles in both the physiology and pathology of the brain. On the one hand, apoptosis is instrumental in the selection of neurons, working coordinately with neuroprotection (16–19). On the other hand, it plays central roles in the development of specific diseases (3). Understanding of factors and mechanisms governing the apoptotic program of nerve cells represents therefore a key issue in modern biomedical sciences.

Among the neuronal pro-apoptotic factors, considerable attention is presently focused on NGF1 and its receptors. In classical studies, this neurotrophin has been shown to afford protection to various families of neurons, working through its high affinity receptor, TrkA. As far as the low affinity NGF receptor, p75NTR, initial studies suggested its role to be cooperative to TrkA (see, for example, Refs. 4 and 5). Recently, however, homologies have been noticed between the transduction domain of p75NTR and those of well known death receptors, i.e. the tumor necrosis factor-α receptor and CD95 (Fas, APO-1) (see Ref. 6). The latter are known to induce apoptosis via complex signal cascades including the generation of a lipid messenger, ceramide, and the proteolytic activation of cysteine proteases, the caspases (7, 8). At the moment, a pro-apoptotic role of p75NTR is supported by results in a variety of systems, including cultured cells as well as knockout and transgenic mice (9–11).

Compared with the other death receptors, p75NTR appears unique inasmuch as only in some cases, apoptosis was shown to develop following NGF binding, whereas in other cases, it occurred in the absence of the specific ligand (spontaneous apoptosis). The signaling events triggered in the first condition are beginning to be elucidated (11–14), whereas those of the second remain undefined. Whatever their nature, these events appear to operate within the cell under the control of various modulators, among which is the short-lived messenger NO (15). Based on results in a number of cell types, from macrophages to neurons, NO was initially suggested to work as a stimulator of apoptosis, whereas recently, it has been shown to play a protective role (16–19).

In this work, the signaling events responsible for p75NTR-induced apoptosis and their regulation by NO were investigated in a panel of transfected SK-N-BE human neuroblastoma clones, some of which had already been shown to undergo spontaneous apoptosis (20). The results demonstrate (i) that both ceramide and caspases play crucial roles in the death signaling cascade induced by p75NTR and (ii) that NO protects against the apoptotic program, working downstream of ceramide generation and upstream of the operative caspase cascade.

EXPERIMENTAL PROCEDURES

Materials—The rabbit polyclonal antibody specific for human p75NTR (9651) was kindly provided by Dr. M. Chao. The rabbit polyclonal

1 The abbreviations used are: NGF, nerve growth factor; NTR, neurotrophin receptor; NO, nitric oxide; AMC, 7-amino-4-methylcoumarin; CMK, chloromethyl ketone; FMK, fluoromethyl ketone; Z-, benzoyloxy carbonyl; SNAP, S-nitrosoacetylpenicillamine; t-N, L-nitro-arginine methyl ester; PBS, phosphate-buffered saline; CHAPS, 3-[3-cholamidopropyl]dimethylammonio]-1-propanesulfonic acid; FACS, fluorescence-activated cell sorter.
antibody specific for human caspase-1 (pan-ICE) and the mouse monoclonal antibody specific for human caspase-3 (clone 10CL19) were purchased from Oncogene Research Products (Cambridge, United Kingdom).

The mouse monoclonal antibody specific for human Bel-2 (clone 100) was from Ancell Corp. (Bayport, MN). The rabbit polyclonal antibody to rabbit Bel-2 (clone 14) was from Sigma (St. Louis, MO).

Clones (see also Bunone et al. (16)) were of the order of 280% in the BEp75B, BEp75H, and BEp75AR clone, were grown in complete medium supplemented with 150 mM NaCl, 15 mM MgCl2, 1 mM EGTA, 50 mM Hepes-KOH, 10% glycerol, and 1% Triton X-100. The reaction mixture was incubated for 1 h at room temperature.

Ceramide phosphate was isolated by thin-layer chromatography (Silica Gel 60) using CHCl3/CH3OH/CH3COOH (65:15.5:5, v/v/v) as solvent and visualized with a ultraviolet lamp. A authentic ceramide 1-phosphate was identified by autoradiography at Rf = 0.25.

Electrophoresis and Western Blot Analysis—Wild-type SK-N-BE cells and clones stably transfected with p75NTR were cultured, harvested using a Pasteur pipette, washed in PBS, and lysed in cell lysis buffer (150 mM NaCl, 15 mM MgCl2, 1 mM EGTA, 50 mM Hepes-KOH, 10% glycerol, and 1% Triton X-100, pH 7.5) supplemented with a protease inhibitor mixture (0.2 mM phenylmethylsulfonyl fluoride, 2 mM benzamidine, and 1 μg/mL o-phenanthroline). After 30–60 min of 4°C rotating device, the cell lysates were centrifuged (for 3 min at 5000 rpm in an Eppendorf microcentrifuge), and the supernatants were assayed for protein content by the bicinchoninic acid procedure.

For electrophoresis and Western blot analyses, protein suspensions were solubilized (10:7 ratio) in SDS sample buffer (48 mM Tris-HCl, pH 6.8, 0.8 M sucrose, 4% SDS, 8% β-mercaptoethanol, and 0.008% bromphenol blue) and heated on a boiling water bath for 5 min. The solubilized proteins were loaded and separated on Laemmli-type SDS-polyacrylamide gels. Protein concentrations were typically 4% in the stacking gel and either 7% or 15% in the running gel for Trk or p75NTR. Bcl-2 and, caspase-1 and -3, respectively. The separated proteins were electrotransferred in a cold room onto a 0.2-mm pore nitrocellulose membrane at a constant 70 mA for 16 h using a buffer containing 25 mM Tris, 192 mM glycine, and 20% methanol, pH 8.3. After brief staining with 0.2% (w/v) Ponceau Red in 3% trichloroacetic acid to reveal the standard molecular mass markers, the blots were blocked with 5% (w/v) low-fat dried milk in PBS supplemented with 0.01% (v/v) Tween 20 (PBS/Tween) and then exposed for 1 h at room temperature to the primary antibody. Blots were washed in 2.5% (w/v) milk in PBS/Tween (5 × 10 min). Immunostaining was then developed by the enhanced chemiluminescence kit, and the relevant bands were revealed by using a Molecular Dynamics Imagequant apparatus.

Caspase Activity Measurements—Samples of 1 × 106 cells were rinsed in cold PBS and lysed in a buffer containing 25 mM Hepes, pH 7.5, 5 mM EDTA, 1 mM EGTA, 5 mM MgCl2, 5 mM dithiothreitol, 1% CHAPS, 10 μg/mL each pepstatin and leupeptin, and 1 mM phenylmethylsulfonyl fluoride. The cell lysates were centrifuged (for 3 min at 5000 rpm in an Eppendorf microcentrifuge), and the supernatants were stored at −80°C until used. Protein content was assayed by the bicinchoninic acid procedure.

Lysates (25 μg of protein) were incubated at 37°C in a buffer containing 25 mM Hepes, pH 7.5, 10% sucrose, 0.1% CHAPS, and 1 mM dithiothreitol supplemented with Ac-DEVD-AMC (50 μM). The increase in fluorescence of the radioactive spots. Authentic ceramide 1-phosphate was identified by autoradiography at Rf = 0.25.
RESULTS

p75NTR Signaling and Cell Death in p75NTR-overexpressing Cell Clones—Receptor-induced apoptosis develops through a series of intracellular events. In addition to (or concomitantly with) the establishment of the death-inducing signaling complex by the recruitment to the plasma membrane receptors of specific cytosolic proteins (see Ref. 7), activation of lipid-specific enzyme(s) occurs, with ensuing generation of metabolites that appear to play a role in the development of the program. In particular, this is the case of ceramide (25–27), a sphingolipid metabolite known to induce apoptosis even when administered to the cells as a synthetic, membrane-permeable analogue. Fig. 1 demonstrates the sensitivity of SK-N-BE cells to one of these analogues, C2-ceramide. The compound was administered in a range of concentrations (1–50 μM) (Fig. 1B), including the range of the endogenous metabolite estimated in our cell clones under resting conditions (5–12 μM) (see also Fig. 2). At all concentrations tested, the lipid metabolite was found to be distinctly more efficacious in the clones stably transfected with p75NTR cDNA than in the wild-type parental neuroblastoma cells (Fig. 1, A and B). Such an increased effect occurred in at least rough proportion to receptor expression: moderate in the cells of the BEp75Hy cell clone (Hy− clone) and much stronger in those of the BEp75B clone (B clone), where expression is ~7-fold higher.

Subsequent experiments were carried out to investigate whether the role of ceramide is only pharmacological or is connected with the regulation of p75NTR-induced apoptosis. Fig. 2A suggests that this may indeed be the case. In fact, compared with wild-type cells, the resting levels of endogenous ceramide were higher in the p75NTR-expressing clones: 50% in the Hy− clone and >100% in the B clone and in the other p75NTR-overexpressing clone employed, BEp75H (H clone). Parallel to the results with endogenous ceramide were those with the cystein proteases known to participate in the apoptotic cascade, i.e., the caspases (7, 8). The activity of the latter enzymes, already conspicuous compared with wild-type cells in the low expression clone, Hy−, reached values 50–85-fold higher in the overexpressing clones, B and H (Fig. 2B). Likewise, DNA laddering (a classical sign of apoptosis) was high (~10-fold of the wild-type value) in clone B, and this value was significantly decreased when cells were pretreated with membrane-permeable caspase inhibitors (Ac-YVAD-OMK, Z-DEVD-FMK, and DEVD-CHO (100 μM), administered either alone or in combination) (Table I and data not shown). In contrast, these peptides had no effect on the cell endogenous ceramide levels (Fig. 2A).

The dependence of ceramide levels and caspase activity on p75NTR was further investigated in the transfected clones by treatment with a natural ligand of the receptor, NGF. For both parameters, this neurotrophin (6 nM) induced significant decreases, which, however, required long periods (tenths of hours) to develop (see Fig. 3 for clone B). Since the employed cells express neither TrkA nor any other Trk receptor, as revealed by Western blots (Fig. 4B) (20), the protective effect of NGF can only be attributed to the expressed p75NTR.

An Apoptosis-resistant p75NTR-transfected Clone—A new clone transfected with the p75NTR cDNA (BEp75AR, indicated below as clone AR) differs profoundly from those described so far (20). The level of p75NTR expression was high in clone AR, comparable to that in clones B and H (Fig. 4A), and Trk receptors were not detectable (Fig. 4B). Yet the AR clone tendency to apoptosis was low, not only under resting conditions, but also after ceramide administration (Fig. 4C, left and middle panels). In contrast, clone AR was as susceptible as the other clones to treatments known to induce apoptosis by non-receptor mechanisms, such as exposure to the Ca2+ ionophore ionomycin (5 μM) (Fig. 4C, right panel; and data not shown). In terms of endogenous ceramide, clone AR resembled the p75NTR-overexpressing clones, B and H, with high resting levels (~11.5 μM), which decreased with time following exposure to NGF (Fig. 4D).

A number of mechanisms that could account for the resistance to apoptosis of the AR clone were investigated. The levels of the apoptosis controller oncogene product, Bcl-2, and of caspases were similar to those found in the other clones (Western blotting) (data not shown). Yet when caspase activity was assayed by the use of a fluorescent substrate, clone AR exhibited very low levels, thus resembling not the other p75NTR-overexpressing clones, but the wild-type parental cells (Fig. 5). Moreover, the caspase activity remained unchanged when clone AR was exposed to exogenous ceramide, a treatment that, even in wild-type cells, induces large increases, exceeding with time the levels observed in the unstimulated p75NTR-overexpressing clones (Fig. 5). From these data, we conclude that, in the AR clone, the tendency to apoptosis induced by p75NTR overexpression is balanced specifically by one (or more) additional event(s) by which the death signaling cascade is blocked upstream of caspase activation.

Role of NO—A series of experiments was carried out to explore the possibility that NO has a role in the regulation of apoptosis in SK-N-BE clones. NO synthase activity was found to vary profoundly in the various clones. Compared with controls, it was clearly increased in the resistant AR clone and greatly reduced in the sensitive clones, especially clones H and B (Fig. 6). In general terms, therefore, this variability correlates inversely to the sensitivity to exogenous ceramide. Exposure to the blocker L-NAME induced large drops in the NO synthase activity, which were parallel in all the preparations investigated (Fig. 6).

Additional evidence for a role of NO was obtained by experiments in which spontaneous apoptosis was significantly reduced in the p75NTR-overexpressing B and H clones by pretreatment with the NO donor SNAP as revealed by various tests, including fluorescein isothiocyanate-annexin V staining and nucleosome accumulation (Table II). The same donor, administered to wild-type cells, was able to prevent (in a dose-dependent fashion) the apoptosis induced by exogenous ceramide (50 μM) (Fig. 7A) and to inhibit the activity of caspases (Fig. 7B). Such a protection was due to NO generation from the donor, and not to other effects of the compound, because it faded out completely when the messenger was trapped by addition of hemoglobin (100 μM) to the cell incubation mixture (Fig. 7A). Likewise, the high caspase resting levels were reduced in the p75NTR-overexpressing cells by the NO donor (Fig. 7C), which, in contrast, failed to modify the high levels of endogenous ceramide (data not shown). As far as the mechanisms by which the protective effects of NO are induced, experiments were carried out in which both wild-type and clone B cells were treated with the membrane-permeable cGMP analogue 8-Br-cGMP (0.5 mM) before exogenous ceramide administration or under resting conditions. Since this treatment did not affect the caspase activity (Fig. 7, B and C) or apoptosis (data not shown), we conclude that one of the classical mediators of NO action, the activation of guanylate cyclase, is most likely not involved in its anti-apoptotic effect.

DISCUSSION

Interest about p75NTR has been greatly stimulated by the conflicting results reported about its function. In cells in which its expression occurs together with that of the other NGF receptor, the tyrosine kinase TrkA, p75NTR has been shown to contribute to the trophic effects induced by ligand binding, playing either a tuning (4, 5, 28, 29) or a true synergistic (30)
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role. Recently, however, p75NTR has been recognized as a death receptor, belonging to the same molecular family as the tumor necrosis factor-α receptor and CD95, and ample evidence of p75NTR-induced apoptosis has been reported (10, 11). Whether the latter effect is induced by the receptor when free or after binding of one of its ligands, such as NGF, remains debated. In two studies in which the binding mode was reported (in oligodendrocytes (Ref. 12; however, see also Ref. 31) and in chick retina neurons (Ref. 13)), the pro-apoptotic activation of p75NTR was shown to trigger the generation of ceramide. This finding is of importance because the lipid derivative, no matter whether generated endogenously or administered exogenously, is known to play a key role in the induction of apoptosis (32,

![Figure 1](image1.png)

**Fig. 1.** Spontaneous and ceramide-induced apoptosis in wild-type SK-N-BE cells and clones expressing different levels of the p75NTR receptor. A, apoptosis in wild-type cells (panel a) and two transfected clones with different levels of p75NTR expression (low levels in clone Hy - (panel b) and high levels in clone B (panel c)) is shown before (insets) and after overnight exposure to exogenous ceramide (50 μM). Apoptotic cells were revealed by FACS analysis. The results shown are representative of three to five experiments. In this and the following figures showing FACS analyses, the apoptotic cell peak is indicated by an arrowhead. B, concentration dependence of apoptosis induced in the indicated SK-N-BE cells by exogenous ceramide, administered as described for A. The arbitrary units on the ordinate refer to absorbance measured at 405 nm. wt, wild-type.

![Figure 2](image2.png)

**Fig. 2.** Endogenous ceramide levels (A) and caspase activity (B) in wild-type SK-N-BE neuroblastoma cells and in various clones expressing different levels of the p75NTR receptor. A, the levels of endogenous ceramide were assayed in the wild-type cells and in three transfected clones in the absence (stippled bars) and presence (black bars) of the caspase inhibitors Ac-YVAD-CMK and Z-DEVD-FMK (100 and 50 μM) as described under "Experimental Procedures." The results shown (means ± S.D.) are representative of three consistent experiments. B, total cell lysates of the wild-type cells and of various p75NTR-transfected SK-N-BE clones were incubated at 37 °C with a 50 μM concentration of the fluorogenic substrate Ac-DEVD-AMC. The fluorescence increase following the cleavage of the AMC residue was monitored for 10 min at 37 °C. Data shown (means ± S.D.) are from the results of five experiments.

![Table 1](image3.png)

**Table 1**

| Treatment       | Released nucleosomes |
|-----------------|----------------------|
| Untreated       | 0.96 ± 0.07          |
| Caspase inhibitors | 0.45 ± 0.07 |
| 24 h            | 0.21 ± 0.04          |
| NGF             | 0.41 ± 0.03          |
| 24 h            | 0.13 ± 0.02          |

Clone B cells were incubated in culture medium supplemented either with caspase inhibitors (Ac-YVAD-CMK together with Z-DEVD-FMK; 50 and 100 μM) or with NGF (6 nM). At the beginning of the incubation as well as 24 and 48 h later, the cells were collected and assayed for internucleosomal degradation of DNA by the Cell Death Detection Elisa Plus kit. The results (means ± S.D. of three different experiments run in triplicate) are expressed as arbitrary units (absorbance at 405 nm/μg of protein).
On the other hand, spontaneous apoptosis taking place in p75<sub>NTR</sub>-expressing cells in the absence of NGF was demonstrated in immortalized neural cells and sensory neurons (34, 35) as well as in the overexpressing SK-N-BE clones employed in this study (20). The intracellular signaling events operative in these various cases were not investigated.

To reconcile these results, a detailed understanding of the p75<sub>NTR</sub> transduction system would be needed. Activation of other death receptors, such as the tumor necrosis factor-α receptor, is known to trigger not a single, but a number of intracellular cascades, only some of which lead to apoptosis, whereas others ultimately induce cell protection and/or proliferation (32, 36, 37). The equilibrium among these various pathways appears to be regulated in a cell type-specific fashion. A task of this work was to elucidate the mechanisms by which p75<sub>NTR</sub> triggers and regulates its spontaneous apoptotic response in SK-N-BE cells. The results we have obtained emphasize the role of both ceramide and caspases. The high levels of endogenous ceramide observed in the p75<sub>NTR</sub>-overexpressing cells were shown to decline, although slowly (24–48 h), after administration of NGF, in parallel with the strong decrease in the probability of apoptosis (20). The possibility that high ceramide levels increase the probability of cells to enter the apoptotic program is confirmed also by their higher sensitivity to the probability of apoptosis (20). The possibility that high ceramide levels increase the probability of cells to enter the apoptotic program is confirmed also by their higher sensitivity to

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verges from the others because of its resistance to apoptosis. Resting clone AR cells are characterized by high ceramide levels and by caspase expression levels comparable to those of the other clones. The signaling difference of clone AR emerged when its low caspase activity failed to rise and its cells failed to enter apoptosis when exposed to exogenous ceramide. Apparently, therefore, the resistance of clone AR is due to a trans-

**TABLE II**

Protective effect of the NO donor SNAP against apoptosis in the p75NTR-overexpressing clones as revealed by two independent procedures

| Treatment | Annexin-positive cells | Histone-associated DNA fragments |
|-----------|------------------------|----------------------------------|
|           | Wild-type | Clone B | Clone H | Wild-type | Clone B | Clone H |
| Untreated | 3.5 ± 1.5 | 85.6 ± 5.3 | 66.2 ± 4.5 | 0.09 ± 0.05 | 0.93 ± 0.08 | 0.88 ± 0.02 |
| + SNAP    | 10.3 ± 2.7 | 59.1 ± 7.9 | 46.0 ± 2.8 | 0.17 ± 0.06 | 0.61 ± 0.05 | 0.52 ± 0.07 |

**FIG. 5.** Caspase activity assayed under resting conditions and upon exogenous ceramide application in wild-type SK-N-BE cells and in the various p75NTR-overexpressing clones. Total cell lysates obtained from either the wild-type (wt) cells or the various p75NTR-transfected SK-N-BE clones, under resting conditions or after exogenous ceramide (50 μM) administration for different times (0, 2, 4, 6, and 16 h), were mixed at 37 °C with a 50 μM concentration of the fluorogenic substrate Ac-DEVD-AMC, and the fluorescence increase following the cleavage of the AMC residue was monitored for 10 min in a spectrofluorometer. The results (means ± S.D.) are from three separate experiments run in duplicate.

**FIG. 6.** NO synthase activity in wild-type SK-N-BE cells and in the various p75NTR-expressing clones. NO synthase (NOS) activity was assayed in the total homogenates of wild-type (wt) SK-N-BE cells and of the various p75NTR-overexpressing clones, prepared both under resting conditions and in the presence of the NO synthase inhibitor L-NAME (500 μM), employed as an internal control. The results shown (means ± S.D.) are from three separate experiments run in triplicate.

**FIG. 7.** Inhibition by the NO donor SNAP of both apoptosis (A) and caspase activity (B and C) in wild-type SK-N-BE (A and B) and clone B (C) cells. A, wild-type SK-N-BE cells were preincubated for 15 min without (panels a and b) or with (panels c and d) the NO donor SNAP (300 μM) in the presence (panel d) or absence (panel c) of the NO scavenger hemoglobin, (100 μM) before receiving, in addition, exogenous ceramide (50 μM, 4 h). C, clone B cells were preincubated for 48 h (changes every 12 h) with either SNAP or 8-Br cGMP at the indicated concentrations before assaying the caspase activity. In both B and C, caspase activity measurements were performed on total cell homogenates by monitoring the cleavage of the fluorogenic substrate Ac-DEVDA-MC. Values are shown as described in the legends to Figs. 2B and 5. The results (means ± S.D.) are from three separate experiments run in duplicate.
duction blockade localized between p75NTR signaling and ceramide generation on the one hand and caspase activation on the other. Such a blockade appears specific for the receptor-initiated pathway since, when other inducing treatments were employed, no difference in apoptosis development was observed between clone AR and the other clones. Of the cell properties that could be possibly responsible for clone AR resistance, some (expression of Trk receptors and increased levels of Bcl-2) could be excluded. A difference detected in clone AR with respect to the other overexpressing clones and also the wild-type SK-N-BE cells was, in contrast, its high NO synthase activity. Thus, NO could have a part in the lack of caspase activation and apoptosis in clone AR cells; whether other, so far unidentified protective factor(s) are also involved remains to be established.

In previous studies, the conclusions about the role of NO in nerve cell apoptosis had been conflicting. In many cases, the messenger was reported to induce (38–40) and in others to protect from (41, 42) programmed cell death. In our work, the NO synthase activity and apoptosis (both spontaneous and induced by ceramide) and (ii) the effects of an NO donor (SNAP) that was able to prevent the ceramide activation of apoptosis in clone AR cells; whether other, so far unidentified protective factor(s) are also involved remains to be established.

In conclusion, the panel of stably transfect SK-N-BE neuroblastoma clones lacking Trk have shown to be interesting models for the study of the signaling and physiological role of the p75NTR receptor. The higher efficacy of the latter in inducing apoptosis in the absence rather than in the presence of its ligand might account for the neurotrophic role attributed in previous studies (30) to the NGF activation of p75NTR working synergistically with TrkA. In other cell types, however, classical apoptotic responses have been triggered following ligand activation of p75NTR (12, 13), as expected for a bona fide death receptor. These apparently conflicting results call attention to the mechanisms that regulate intracellularly the development of the apoptotic program. In this study on p75NTR-expressing SK-N-BE clones, the event that seems to play a major role is the generation of NO. In fact, high levels of the gaseous messenger inhibit the activation of caspasases by a mechanism that appears to be cGMP-independent. Therefore, in the case of p75NTR-induced apoptosis, at least two levels of control appear to be functioning: at the receptor, with its alternative free/bound signaling; and within the cytoplasm, where multiple signals, including those triggered by NO, appear to participate in the regulation of the life-or-death decision (11).

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