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NEUROTENSIN STIMULATES FORMATION OF CYCLIC GMP IN MURINE NEUROBLASTOMA CLONE N1E-115

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Neurotensin is an endogenous tridecapeptide (pGlu-Leu-Tyr-Glu-Asn-Lys-Pro-Arg-Arg-Pro-Tyr-Ile-Leu) found primarily in the brain and gastrointestinal system with a wide range of reported biological activities (see reviews of Bissette et al., 1978, and Miller, 1981). Following intravenous challenge with neurotensin, effects occurring in the mammalian gastrointestinal tract include a decrease in gastrointestinal motility, an increase in blood flow, a decrease in gastric acid secretion, and hyperglycemia. Vascular effects following neurotensin administration include vasodilation, hypotension, and increased permeability of blood vessels. In addition, neurotensin is considered a possible neurotransmitter in the central nervous system and is known to play a role in controlling body temperature and nociception.

Receptors for neurotensin have been demonstrated in brain synaptosomal membranes, intestinal smooth muscle, and mast cells. However, a biochemical event associated with activation of these receptors has not been known. Here we report preliminary results that a widely studied clone of murine neuroblastoma (N1E-115) contains receptors for neurotensin and that activation of these receptors results in a marked increase in cyclic GMP synthesis.

Clone N1E-115 was cultured as described by Gilbert et al. (1982). The formation of intracellular cyclic GMP was measured by the method of Richelson et al. (1978), in which cyclic [^{3}H]GMP was isolated chromatographically from cells labeled with radioactive precursor prior to neurotensin stimulation. The only modifications to the procedure as described were omitting sonication of each reaction well prior to chromatography, rinsing individual wells after sample application to Dowex columns, and including routinely the precipitation step subsequent to column fractionation.

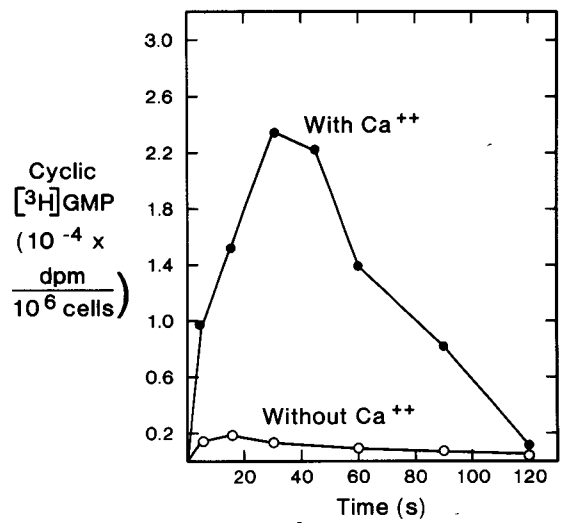

Fig. 1. Time course of cyclic [^{3}H]GMP stimulation in neuroblastoma clone N1E-115 by neurotensin. Intact N1E-115 cells (passage number 9; 7 days after subculture; 100000 cells/well) were stimulated after prelabeling with [^{3}H]guanine sulfate for increasing lengths of time with 0.1 μM neurotensin. The cyclic [^{3}H]GMP formed was isolated by the procedure of Richelson et al. (1978) as described in the text. Incubation medium was a phosphate buffered saline solution 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 mOsm) (●———●). Incubation medium described was modified to contain 2.0 mM $MgCl_2$, 1.0 mM EGTA, and 0 mM $CaCl_2$ (○———○). Maximum stimulation in the presence of Ca^{2+} was ~19 fold over basal. These data represent one of three similar experiments.

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Neurotensin (Boehringer Mannheim, Indianapolis, IN) stimulated the production of intracellular cyclic GMP 10 ± 3 fold over basal (mean $\pm$ S.E.; $n = 5$) in N1E-115 cells in a dose related fashion with an ED_{50} of 13 ± 5 nM (mean $\pm$ S.E.; $n = 5$). As shown in fig. 1, the formation of cyclic [^{3}H]GMP reached a maximum 30 s after stimulation of the clone with neurotensin and required the presence of Ca^{2+} in the incubation medium. Neurotensin-(8-13) (Bachem, Torrance, CA), the carboxyl-terminal portion of neurotensin, was more potent than neurotensin in stimulating the formation of cyclic [^{3}H]GMP, with 7 ± 3 fold production over basal (mean $\pm$ S.E.; $n = 3$) and an ED_{50} of 0.34 ± 0.13 nM (mean $\pm$ S.E.; $n = 3$). Neurotensin-(1-8) (Sigma, St. Louis, MO), the amino-terminal portion of neurotensin, had absolutely no effect on cyclic [^{3}H]GMP production, even at concentrations as high as 0.1 mM (3 experiments). Preliminary binding studies have demonstrated saturable, specific binding of [^{3}H]neurotensin (New England Nuclear, Boston, MA) to intact N1E-115 cells in the concentration range of 1-16 nM. This binding displayed linearity with cell number/tube.

Clone N1E-115 has many biochemical, electrophysiological, and anatomical properties of adrenergic neurons and contains receptors for several putative neurotransmitters (e.g., muscarinic acetylcholine, histamine H_1) and neuromodulators (e.g., δ opioid). Recently, this laboratory has demonstrated that these cells contain receptors for thrombin (Snider and Richelson, 1983) and bradykinin (Snider and Richelson, submitted for publication). Activation of either of these receptors increases intracellular cyclic GMP synthesis with a time course and calcium dependency essentially identical to that for neurotensin. In intact cell binding assays with [^{3}H]bradykinin, neurotensin demonstrated no competition with that radioligand (Snider, R.M., personal communication).

The cyclic GMP results with neurotensin are in accordance with studies relating the structure of this peptide and its analogs and fragments to biological activity (Bissette et al., 1978). The carboxyl-terminal portion of neurotensin is almost as effective as the parent protein for all described biological activities to date, and is apparently requisite for receptor interaction. Neurotensin-(1-10) is reported to be inactive biologically. The data presented here also give support to the hypothesis that neurotensin is a neurotransmitter or neuromodulator since these receptors are present on a neuronal cell type and as cyclic nucleotides are generally considered second messengers of neurotransmitters in the mammalian nervous system.

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