Biochemical Pharmacology, Vol. 36, No. 6, pp. 869–874, 1987.
Printed in Great Britain.

0006-2952/87 $3.00 + 0.00

COMPARISON OF THE STIMULATION OF INOSITOL PHOSPHOLIPID HYDROLYSIS AND OF CYCLIC GMP FORMATION BY NEUROTENSIN, SOME OF ITS ANALOGS, AND NEUROMEDIN N IN NEUROBLASTOMA CLONE N1E-115

KIYOKO S. KANBA and ELLIOTT RICHELSON*
Departments of Psychiatry and Pharmacology, Mayo Clinic and Foundation, Rochester, MN 55905, U.S.A.

(*Received* 3 *April* 1986; *accepted* 12 *August* 1986)

Abstract—Neurotensin, some of its analogs, and neuromedin N were examined for comparison of their potencies at stimulating inositol phospholipid hydrolysis and cyclic GMP synthesis in intact murine neuroblastoma cells (clone N1E-115). Neurotensin(8-13) and acetylneurotensin(8-13) had the highest potencies for the stimulation of the hydrolysis of inositol phospholipid, which were about three times as potent as neurotensin (EC_{50} = 0.9 nM). On the other hand, fragments of the amino-terminal portion of neurotensin, such as neurotensin(1-6), neurotensin(1-8) and neurotensin(1-11), showed no ability to stimulate this hydrolysis. Neuromedin N, which is similar in structure to neurotensin(8-13) and which has been demonstrated to stimulate cyclic GMP formation [J. A. Gilbert and E. Richelson, *Eur. J. Pharmac.* **129**, 379 (1986)], had EC_{50} values of 2.5 and 4.5 nM for release of [^{3}H]inositol phosphates and stimulation of cyclic [^{3}H]GMP respectively. A strong correlation was obtained between the EC_{50} values for neurotensin and several analogs in the stimulation of the release of inositol phosphates and the EC_{50} values for these peptides in the stimulation of cyclic GMP formation in neuroblastoma clone N1E-115 cells under similar experimental conditions. Thus, these two different biochemical effects of neurotensin and its analogs appear to be mediated by the same receptor site, which may also have been the site of action of neuromedin N in these cells.

Neurotensin (pGlu-Leu-Tyr-Glu-Asn-Lys-Pro-Arg-Arg-Pro-Tyr-Ile-Leu), a tridecapeptide first discovered in bovine hypothalamus [1], is a putative neurotransmitter and has various biological activities [2, 3] in the central nervous system. In neuroblastoma clone N1E-115 cells [4] and in rat cerebellar slice [5], neurotensin stimulates the formation of intracellular cyclic GMP, which is generally thought to be a second messenger of some neurotransmitters.

Many structure–activity studies of neurotensin have been done using various analogs in various species in terms of receptor binding [6–8], pharmacology [9, 10], electrophysiology [11], and behavior [12]. For several neurotensin analogs, this laboratory has reported a strong correlation of the binding affinity with the ability to stimulate the formation of intracellular cyclic GMP in neuroblastoma clone N1E-115 cells [13]. The binding of these analogs has also been demonstrated in human frontal cortical membranes [14]. These structure–activity studies indicate the importance of the carboxy-terminal portion of neurotensin for both binding and biological activities.

Recently, some studies showed that neurotensin stimulates inositol phospholipid metabolism in rat brain slices [15], cultured rat anterior pituitary cells [16], and neuroblastoma clone N1E-115 cells [17]. Although it has been reported that many neurotransmitters which stimulate cyclic GMP formation also stimulate inositol phospholipid hydrolysis [18], the relationship between this hydrolysis and cyclic GMP formation is still unclear. Therefore, we determined the EC_{50} values for neurotensin and several of its analogs at stimulating inositol phospholipid hydrolysis and at stimulating intracellular cyclic GMP formation under very similar experimental conditions, using equally divided daughter cells of neuroblastoma clone N1E-115. We also compared the activities of neuromedin N (Lys-Ile-Pro-Tyr-Ile-Leu), an endogenous hexapeptide which was isolated from the porcine spinal cord by Minamino *et al.* [19], since its has the same four amino acid residues at the carboxy-terminal portion end as does neurotensin. Our results show a strong correlation between the EC_{50} values determined in each assay for these peptides.

In addition, we present some results with peptidase inhibitors. These compounds were studied in an effort to achieve experimental conditions allowing us to assay release of inositol phosphates and cyclic GMP for identical time periods without the confounding variable of the degradation of the peptide agonists.

METHODS

Cell culture. Culture of murine neuroblastoma clone N1E-115 cells was achieved as described previously [13] using Dulbecco-Vogt's modification of Eagle's medium (GIBCO, Grand Island, NY) without antibiotics and supplemented with 10% (v/v)

*To whom correspondence may be addressed.

fetal bovine serum (GIBCO). Cells of passage numbers 9–18 were used for these experiments. Subculture was achieved by aspirating medium and then incubating for 10 min at 37° in 10 ml of modified Puck's D_1 solution. Cells ($4–8 \times 10^5$) were inoculated into flasks on day 0. The culture medium was changed daily beginning 6 days 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, 9–23 days after subculture for both cyclic GMP assays and inositol phosphate assays.

Assay for the formation of cyclic [^{3}H]*GMP.* The assay used for measurement of intracellular cyclic GMP formation was essentially the same as described previously [13, 20], in which cyclic [^{3}H]GMP formed in clone N1E-115 cells was isolated chromatographically from cells labeled with radioactive precursor prior to receptor stimulation. Harvested cells were resuspended in 10 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 mOsm). Cells were again collected by centrifugation (300 *g*, 1 min), resuspended in 2 ml of solution I and transferred to a 25-ml Erlenmeyer flask. Twenty microliters of the suspension was removed for enumeration of cells with an electronic cell counter (Coulter Electronics, Hialeah, FL). For experiments comparing the EC_{50} values for peptides at stimulating cyclic GMP formation with their respective EC_{50} values at stimulating inositol phosphate release, the cellular pellet was resuspended in 4 ml of solution I and divided equally between two 25-ml Erlenmeyer flasks: one group for use in the cyclic GMP assay, the other for use in the inositol phosphate assay.

To the 2-ml cell suspension was added 20 μCi of [^{3}H]guanosine (5 Ci/mmole, ICN Radiochemicals, Irvine, CA) for radioactively labeling pools of GTP. After a 45-min incubation at 37° in a shaking bath at 80 oscillations/min, cells were collected by two short centrifugations (Beckman, microfuge B) and were resuspended in a volume of solution I to give 10^5 cells/270 μl. Two hundred and seventy microliters of cell suspension was distributed to each well of a Linbro multiwell plate (Flow Laboratories, McLean, VA), and incubated for 20 min at 37° at 60 oscillations/min. To cells in individual wells was added 30 μl of solution I (to obtain basal cyclic [^{3}H]GMP values) or various concentrations of peptides in this solution. After 1.5 min, which provided maximal levels of formation of cyclic [^{3}H]GMP by neurotensin, each reaction was terminated by adding 30 μl of 50% (w/v) trichloroacetic acid solution.

Assay for the release of [^{3}H]*inositol phosphates.* Cells were prepared with the use of the same procedure as for the cyclic [^{3}H]GMP assay and incubated with 40–50 μCi of myo-[^{3}H]inositol (15 Ci/mmole, American Radiolabelled Chemicals, St. Louis, MO) for 60 min at 37° in a shaking water bath at 80 oscillations/min. Cells were collected by two short centrifugations and were resuspended in a volume of solution I to give 10^5 cells/240 μl. Two-hundred and forty microliters of cell suspension was dispensed into 12 × 75 mm glass tubes and incubated for 30 min at 37° with 30 μl of 10 mM LiCl. To cells in tubes was added 30 μl of solution I (to obtain basal [^{3}H]inositol phosphate values) or various concentrations of peptides in this solution and cells were further incubated for 10 min at 37°. Each reaction was stopped with the addition of 750 μl of cold chloroform–methanol (1:2, v/v). Each tube was then vortexed and placed on ice. The phases were formed by adding 250 μl of chloroform and 250 μl (1200 dpm) of an aqueous solution of L-myo-[^{14}C]inositol 1-phosphate (50 mCi/mmole, Amersham) as an internal standard. After further vortexing, the tubes were centrifuged at 400 *g* for 5 min. Six hundred microliters of the upper phase was transferred to a 12 × 75 mm polystyrene tube (Sarstedt, West Germany) in which 2 ml of water was contained. This solution was applied to an AG 1-X8 resin (0.8 × 1.5 cm, 100–200 mesh, formate form, Bio-Rad Laboratories, Richmond, CA) and then washed with 20 ml of 60 mM ammonium formate/5 mM sodium tetraborate. Inositol phosphates were eluted with 4 ml of 1 M ammonium formate/100 mM formic acid and collected into a 12 × 75 mm polystyrene tube. Each eluate was decanted into a plastic scintillation vial followed by addition of 16 ml of Safety-Solve (RPI, Mount Prospect, IL). Radioactivity was measured in a Beckman liquid scintillation counter. Data were corrected for recovery of internal standard which averaged about 58%.

Materials. Neurotensin was purchased from Boehringer–Mannheim (Indianapolis, IN). Neuromedin N and acetylneurotensin(8-13) were purchased from Peninsula Lab Inc. (Belmont, CA). Neurotensin(1-8), neurotensin(1-11), [Gln^4]neurotensin and *o*-phenanthroline were obtained from the Sigma Chemical Co. (St. Louis, MO). Neurotensin(8-13) and neurotensin(9-13) were supplied from Bachem (Torrance, CA); z-pro-prolinal was provided by Dr. Sherwin Wilk, Department of Pharmacology, Mount Sinai School of Medicine, New York.

RESULTS

With different lengths of incubation required for the two different biochemical assays, we wanted to prevent peptide degradation which alone could potentially affect EC_{50} values. Therefore, we tested a combination of 1 mM *o*-phenanthroline and 1 μM z-pro-prolinal [21]. A similar combination was reported previously to inhibit breakdown of neurotensin while having no effect on the cyclic GMP response [22].

When this combination of inhibitors was added at the same time as neurotensin, the stimulation of cyclic [^{3}H]GMP was inhibited by up to 70% (Fig. 1). *o*-Phenanthroline (1 mM) alone when preincubated for 30 min prior to addition of neurotensin gave similar inhibition of the formation of cyclic [^{3}H]GMP (data not shown). Therefore, we abandoned the use of these inhibitors.

Effect of incubation time with neurotensin on its EC_{50} *for stimulation of the release of* [^{3}H]*inositol phosphates.* Maximal responses for neurotensin in the cyclic GMP assay and in the inositol phosphate

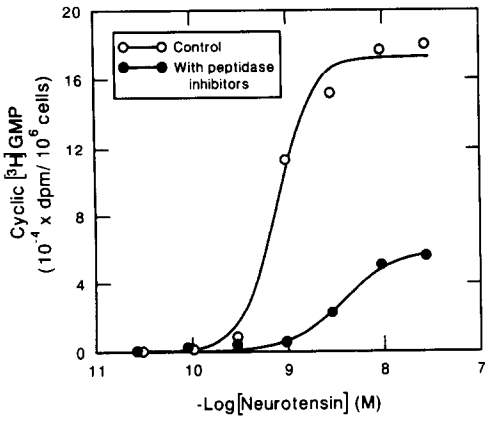

Fig. 1. Effect of the combination of 1 mM *o*-phenanthroline and 1 μM z-pro-prolinal on the stimulation of intracellular cyclic [^{3}H]GMP formation by neurotensin in neuroblastoma clone N1E-115. Cells were stimulated at the indicated concentrations of neurotensin for 1.5 min in the presence (●—●) or absence (○—○) of 1 mM *o*-phenanthroline and 1 μM z-pro-prolinal which were added at the same time as neurotensin. Details of the procedure are described in Methods. A typical experiment in which passage 15 cells (16 days after subculture) were used is shown. Similar results were obtained in one other experiment. Each point represents the mean of triplicate samples from which the mean of triplicate basal values has been subtracted.

assay were obtained at 1.5 and 10 min respectively. This value for the cyclic GMP assay is longer than the one this laboratory reported previously [4], a result which may reflect differences in the type of serum used to culture cells (fetal in the present study vs newborn in the earlier study). As shown in Fig. 2, stimulation of cells with neurotensin in the inositol phosphate assay for 1.5 or 10 min yielded EC_{50} values

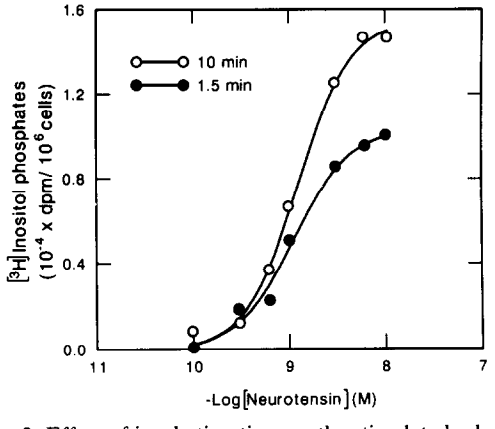

Fig. 2. Effect of incubation time on the stimulated release of [^{3}H]inositol phosphates by neurotensin in neuroblastoma clone N1E-115 cells. Cells were stimulated by the indicated concentrations of neurotensin for 1.5 min (●—●) and 10 min (○—○) in the presence of 10 mM LiCl as described in Methods. A typical experiment in which passage 10 cells (15 days after subculture) were employed is shown. Similar results were obtained from three independent experiments. Each point represents the mean of triplicate samples from which the mean of triplicate basal values has been subtracted.

of 1.1 and 1.3 nM respectively. As expected, the efficacy for neurotensin with a 1.5-min incubation was much less than (60%) that with 10 min. Therefore, for subsequent experiments we chose 1.5 min as the incubation time for the cyclic GMP assays and 10 min for inositol phosphate assays. The only other difference between the two assays was that 10 mM LiCl was present for the inositol phosphate assays, but absent in the cyclic GMP assays since Li^+ inhibits receptor-mediated cyclic GMP synthesis [23, 24].

Correlation of the stimulation of the release of [^{3}H]inositol phosphates with the stimulation of the formation of intracellular cyclic [^{3}H]GMP by neurotensin and related peptides. Neurotensin and related peptides (see Table 1 for structures), including neuromedin N (Fig. 3A), stimulated the accumulation of [^{3}H]inositol phosphates 1- to 2-fold over basal values in neuroblastoma clone N1E-115 cells. On the other hand, neurotensin and these other peptides, including neuromedin N (Fig. 3B), stimulated the formation of cyclic [^{3}H]GMP 5- to 14-fold over basal values. Therefore, the apparent efficacies for neurotensin and its analogs in stimulating the release of [^{3}H]inositol phosphates was more than ten times less than that in stimulating the formation of cyclic [^{3}H]GMP.

Table 2 lists the EC_{50} values for neurotensin and related peptides determined in the two different biochemical assays using daughter cells of neuroblastoma clone N1E-115 cells. Neurotensin(8-13) and acetylneurotensin(8-13) were about three times more potent than neurotensin itself in these assays. Amino-terminal fragments of neurotensin, such as neurotensin(1-6), neurotensin(1-8) and neurotensin(1-11), showed no response at 10 μM or less, whereas carboxy-terminal fragments of neurotensin were quite potent. A strong correlation ($r^2 = 0.97$) was obtained between the EC_{50} values for the stimulation of the release of [^{3}H]inositol phosphates and the EC_{50} values for the stimulation of the formation of intracellular cyclic [^{3}H]GMP by these peptides (Fig. 4).

DISCUSSION

This paper presents the results of studies testing the potencies of neurotensin and related peptides at stimulating two different biochemical events in murine neuroblastoma cells (clone N1E-115), namely, release of inositol phosphates and cyclic GMP formation. The EC_{50} values for each peptide in the two assays were very similar (Table 2), a result which strongly suggests that these two biochemical events are mediated by the same receptor.

Ideally, for comparison of EC_{50} values in two different biochemical events, cells should be studied under identical conditions. With the peptides under study, we could not quite achieve this ideal because of the need to use a longer incubation time (10 min) as well as 10 mM LiCl in the inositol phosphate assay in order to obtain sufficiently high levels of released [^{3}H]inositol phosphates. Previously, we showed that Li^+ non-competitively inhibits cyclic GMP formation stimulated by neurotensin with an IC_{50} of 14 mM [24]. Thus, the use of Li^+ in the cyclic GMP assay was precluded by the fact that 10 mM Li^+ reduces the

Table 1. Structures of neurotensin and related peptides

Position/Peptide	1	2	3	4	5	6	7	8	9	10	11	12	13
Neurotensin	pGlu	Leu	Tyr	Glu	Asn	Lys	Pro	Arg	Arg	Pro	Tyr	Ile	Leu
[Gln^4]neurotensin	pGlu	Leu	Tyr	Gln	Asn	Lys	Pro	Arg	Arg	Pro	Tyr	Ile	Leu
Neurotensin(8-13)								Arg	Arg	Pro	Tyr	Ile	Leu
Acetylneurotensin(8-13)								Acetyl-Arg	Arg	Pro	Tyr	Ile	Leu
Neuromedin N								Lys	Ile	Pro	Tyr	Ile	Leu
Neurotensin(9-13)									Arg	Pro	Tyr	Ile	Leu
Neurotensin(1-6)	pGlu	Leu	Tyr	Glu	Asn	Lys							
Neurotensin(1-8)	pGlu	Leu	Tyr	Glu	Asn	Lys	Pro	Arg					
Neurotensin(1-11)	pGlu	Leu	Tyr	Glu	Asn	Lys	Pro	Arg	Arg	Pro	Tyr		

response by about one-half, although the EC_{50} for the peptide does not change.

In the presence of intact clone N1E-115 cells, [^{3}H]neurotensin (0.1 μM) was degraded nearly completely by 5 min, and the rate of degradation of [^{3}H]neurotensin(8-13) (0.1 μM) was much faster [25]. Thus, it was important to be concerned about degradation of peptides, especially when longer incubation times were employed. Therefore, we made attempts to minimize the degradation of peptides with the use of peptidase inhibitors. These were abandoned when we found marked inhibition of receptor-mediated cyclic GMP synthesis by *o*-phenanthroline. We did show, however, that with a 10-min incubation time, the EC_{50} for release of inositol phosphates by neurotensin was not different from that obtained at 1.5 min (Fig. 2), the incubation time used for the cyclic GMP assay.

The strong correlation of the EC_{50} data indicate that the structure–activity requirements for the two responses were similar, if not identical. Thus, in both assays the carboxy-terminal end of the molecule was essential for activity (Table 2). This laboratory has shown previously the requirement for the carboxy-terminal end of neurotensin for binding of [^{3}H]neurotensin and stimulation of cyclic GMP in these cells [13]. Amar *et al.* [22] also using N1E-115 cells found a strong correlation between the affinity of binding of [^{125}I-Tyr^3]neurotensin and the potency of stimulation of cyclic GMP formation for neurotensin and three of its analogs. In addition, Canonico *et al.* [16] measuring stimulation of phospholipid hydrolysis in rat anterior pituitary cells found results compatible with our findings for neurotensin and a few related peptides. Others have also found similar structural requirements for effects of these peptides in other systems of various species [9–12].

Neuromedin N, an endogenous hexapeptide first discovered in porcine spinal cord [19] (see Table 1 for structure), has the identical four carboxy-terminal amino acids as does neurotensin. Some studies [26, 27] have suggested that neuromedin N is a mammalian form of the avian [Lys^8-Asn^9]neurotensin(8-13) which was isolated from chicken intestine and brain [28, 29]. However, no study has yet answered the question of whether neuromedin N has its own receptor. We showed in the previous study [30] and in this study (Fig. 3B) that neuromedin N stimulated cyclic GMP formation in N1E-115 cells with high potency (Table 2). This study is the first report of

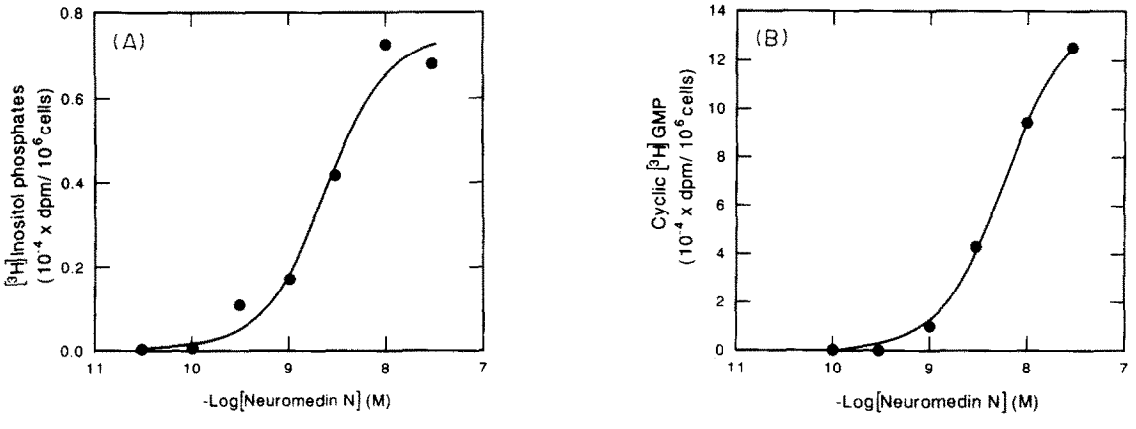

Fig. 3. (A) Stimulation of the release of [^{3}H]inositol phosphates by neuromedin N in intact neuroblastoma clone N1E-115 cells. Cells were incubated with the indicated concentrations of neuromedin N for 10 min in the presence of 10 mM LiCl. (B) Stimulation of the formation of cyclic [^{3}H]GMP by neuromedin N in intact neuroblastoma clone N1E-115 cells. Cells were incubated with the indicated concentrations of neuromedin N for 1.5 min. Both figures are representative results from one of three independent experiments. Cells (passage 12, 15 days after subculture) were equally divided into 2, one half for the inositol phosphate assay (A), the other half for the cyclic GMP assay (B). Each point represents the mean of triplicates from which the mean of triplicate basal values has been subtracted.

Table 2. Potencies of neurotensin and related peptides at biochemical activities in intact clone N1E-115 cells

Peptide	Stimulation of release of [^{3}H]inositol phosphates EC_{50} (nM)	Stimulation of cyclic [^{3}H]GMP formation EC_{50} (nM)	Number of experiments
Neurotensin(8-13)	0.27 ± 0.05	0.34 ± 0.07	4
Acetylneurotensin(8-13)	0.33 ± 0.07	1.0 ± 0.2	4
[Gln^4]neurotensin	0.4 ± 0.1	0.64 ± 0.06	3
Neurotensin	0.9 ± 0.1	1.2 ± 0.3	9
Neuromedin N	2.5 ± 0.3	4.5 ± 0.9	3
Neurotensin (9-13)	21 ± 1	40 ± 10	3
Neurotensin(1-6)	NR*	NR*	2
Neurotensin(1-8)	NR*	NR*	2
Neurotensin(1-11)	NR*	NR*	2

The EC_{50} values for the stimulation of the release of [^{3}H]inositol phosphates and the formation of intracellular cyclic [^{3}H]GMP for neurotensin and related peptides were obtained using equally divided daughter cells of neuroblastoma clone N1E-115 under the same experimental conditions with the exception that in the inositol phosphate assay 10 mM LiCl was present and the incubation time was 10 min instead of 1.5 min. Each EC_{50} represents the mean ± SEM of two to nine independent experiments each performed in triplicate.

* No response at 10 μM.

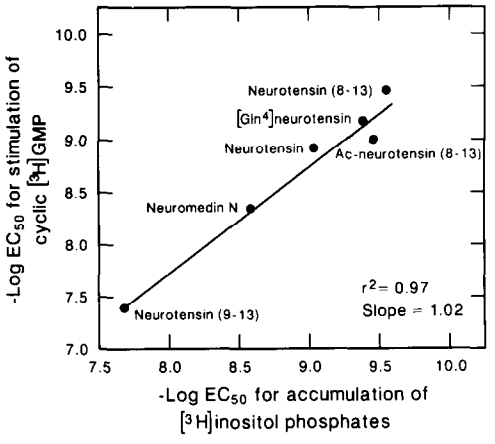

Fig. 4. Correlation between the EC_{50} for the stimulation of the accumulation of [^{3}H]inositol phosphates and the EC_{50} for the stimulation of the formation of intracellular cyclic [^{3}H]GMP for neurotensin and related peptides in neuroblastoma clone N1E-115 cells.

the stimulated release of inositol phosphates by neuromedin N in tissue (Fig. 3A). Whether these biochemical events mediated by neuromedin N result from activation of the neurotensin receptor or its own receptor is undergoing further study.

Acknowledgements—The authors thank Doctor S. Wilk, Department of Pharmacology, Mount Sinai School of Medicine, New York, for the gift of z-pro-prolinal. We also thank Michael Pfenning for technical advice. This work was supported by the Mayo Foundation and Grant MH27692 from the U.S.P.H.S. (N.I.M.H.).

REFERENCES

1. R. Carraway and S. E. Leeman, *J. biol. Chem.* **248**, 6854 (1973).
2. R. Quirion, *Peptides* **4**, 609 (1983).
3. C. B. Nemeroff, P. W. Kalivas, R. N. Golden and A. J. Prange, Jr., *Pharmac. Ther.* **24**, 1 (1984).
4. J. A. Gilbert and E. Richelson, *Eur. J. Pharmac.* **99**, 245 (1984).
5. J. A. Gilbert, M. McKinney and E. Richelson, *Soc. Neurosci. Abstr.* **10**, 378 (1984).
6. P. Kitabgi, R. Carraway, J. Van Rietschoten, G. Granier, J. L. Morgat, A. Menez, S. Leeman and P. Freychet, *Proc. natn. Acad. Sci. U.S.A.* **74**, 1846 (1977).
7. M. Goedert, P. Pittaway, B. J. Williams and P. C. Emson, *Brain Res.* **304**, 71 (1984).
8. C. Granier, J. Van Rietschoten, P. Kitabgi, C. Poustis and P. Freychet, *Eur. J. Biochem.* **124**, 117 (1982).
9. R. Quiron, F. Rioux, D. Regoli and S. St.-Pierre, *Eur. J. Pharmac.* **66**, 257 (1980).
10. R. Kerouac, S. St.-Pierre and F. Rioux, *Peptides* **5**, 695 (1984).
11. F. Baldino, Jr., L. G. Davis and B. Wolfson, *Brain Res.* **342**, 266 (1985).
12. F. B. Jolicoeur, A. Barbeau, F. Rioux, R. Quirion and S. St.-Pierre, *Peptides* **2**, 171 (1981).
13. J. A. Gilbert, C. J. Moses, M. A. Pfenning and E. Richelson, *Biochem. Pharmac.* **35**, 391 (1986).
14. K. S. Kanba, S. Kanba, H. Okazaki and E. Richelson, *J. Neurochem.* **46**, 945 (1986).
15. M. Goedert, R. D. Pinnock, C. P. Downes, P. W. Mantyh and P. C. Emson, *Brain Res.* **323**, 193 (1984).
16. P. L. Canonico, M. A. Sortino, C. Speciale and U. Scapagnini, *Molec. cell. Endocr.* **42**, 215 (1985).
17. R. M. Snider, C. Forray, M. Pfenning and E. Richelson, *J. Neurochem.* **47**, 1214 (1986).
18. M. J. Berridge, *Molec. cell. Endocr.* **24**, 115 (1981).
19. N. Minamino, K. Kangawa and H. Matsuo, *Biochem. biophys. Res. Commun.* **122**, 542 (1984).
20. E. Richelson, F. G. Prendergast and S. Divinetz-Romero, *Biochem. Pharmac.* **27**, 2039 (1978).
21. S. Wilk, T. C. Friedman and T. B. Kline, *Biochem. biophys. Res. Commun.* **130**, 662 (1985).
22. S. Amar, J. Mazella, F. Checler, P. Kitabgi and J-P. Vincent, *Biochem. biophys. Res. Commun.* **129**, 117 (1985).
23. S. Kanba, M. Pfenning and E. Richelson, *Psychopharmacology* **86**, 413 (1985).

24. S. Kanba, M. Pfenning, K. S. Kanba and E. Richelson, *Eur. J. Pharmac.* **126**, 111 (1986).
25. J. A. Gilbert and E. Richelson, *Soc. Neurosci. Abstr.* **12**, 762 (1986).
26. A. Reiner and R. E. Carraway, *Brain Res.* **341**, 365 (1985).
27. M. Goedert, W. N. Schwartz and B. J. Williams, *Brain Res.* **342**, 259 (1985).
28. R. E. Carraway and C. F. Ferris, *J. biol. Chem.* **258**, 2475 (1983).
29. R. E. Carraway, S. E. Ruane and R. S. Ritsema, *Peptides* **4**, 111 (1983).
30. J. A. Gilbert and E. Richelson, *Eur. J. Pharmac.* **129**, 379 (1986).