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DISCUSSION

DR. HAILE T. DEBAS (San Francisco, California): I believe Drs. Evers, Townsend, and Thompson asked me to discuss this paper in the hope, rather than the belief, they would nudge me into the new era of molecular biology. I have to assure them that they failed in that effort.

This group is to be congratulated for its success in developing this unique human carcinoid cell line. Having shown that there is cyclic and mediated transcription of regulation of neurotensin, they are now poised to ask the important questions of what happens proximal and distal to the forskolin step. Specifically is the initiation of the intracellular process purely dependent on adenylate cyclase activation or are other regulatory membrane proteins involved?

And more importantly, how is the signal transferred to the nucleus for transcription to occur? If there is autocrine growth regulation by neurotensin, is this mediated by a different pathway; specifically are proto-oncogenes involved?

I have two questions for Dr. Evers. First you have shown increased message in response to forskolin, but the important issue is showing translation. Specifically how well did the increase in neurotensin messenger RNA correlate, both temporarily and in the dose-response relationship, to the secretion of neurotensin as measured by RIA?

Second you have shown that neurotensin is transcribed in two messenger RNAs. Do you think this is specific splicing that might have functional significance? If so could you hypothesize on the significance of the two messenger RNAs?

Thank you.

DR. DANA K. ANDERSEN (Chicago, Illinois): This laboratory has established a tradition at this meeting of providing state-of-the-art information on the biology and surgical significance of gastrointestinal endocrinology, and this paper certainly extends this tradition.

Perhaps the real significance of this work is the totality of the accomplishment. A portion of a metastatic tumor was transferred to a stable *in vivo* system, which allows the examination of mechanisms responsible for tumor function. The expression of specific genes, such as the neurotensin-neuromedin-C gene, has been quantified, and specific agents that promote or suppress the expression of these genes can be identified. This is an outstanding accomplishment.

Gene expression, or the transcription of mRNA from the gene, is a vital step in our understanding of the function of the tumor cell because the mere measurement of the final secretory product, in this case neurotensin, fails to provide a complete picture of the function of the cell. For example this study shows that serotonin suppresses the expression of the neurotensin gene. But this fact would go unnoticed if we relied on the measurement of neurotensin alone, as little neurotensin secretion was observed under basal circumstances.

That being the case, my first question is whether the authors could detect a reduction in cellular neurotensin content coincident with the

suppression of neurotensin mRNA, and if not, whether they can provide any corroborative evidence that this suppression has any functional outcome on the secretory or growth activities of this tumor?

Because the tumor is a foregut carcinoid that produces serotonin, my second question is whether the tumor tonically suppresses neurotensin gene expression because of its own endogenous serotonin synthesis? Are the levels of serotonin administered to the cell culture similar to the levels of serotonin one might expect in the intracellular or extracellular compartments of the tumor? Does ongoing synthesis and secretion of serotonin by the tumor result in permanent suppression of neurotensin gene expression and synthesis of neurotensin by the tumor? If so one wonders why or how neurotensin is produced at all by this tumor.

This study corroborates the finding that expression of the neurotensin gene is related to a cyclic AMP-dependent element of the gene itself. One wonders, however, whether the expression of a whole host of genes is not stimulated by raising endogenous cyclic AMP levels by the addition of forskolin. Is there some specificity for synthetic processes related to secretory as opposed to growth responses of the tumor?

Finally although the data show recovery of the mRNA transcription stimulated by forskolin back to basal levels, and the authors conclude that induced transcription therefore is time limited, I would ask also whether this recovery might not simply be due to decreasing availability of cyclic AMP produced by forskolin?

DR. JOHN NIEDERHUBER (Baltimore, Maryland): The task before this particular investigator and his group is to use a specific cell line to look at signal transduction pathways and to ask specific questions about a given gene that they have identified as potentially involved in this process. Their goal is to study the expression of this gene and to characterize the resultant peptide product of the gene.

I think you have gathered from the comments of the previous two discussants that these are complex and difficult questions.

The obvious cautions with such a model, of course, need to be noted. Their model involves the use of a transformed cell line. As the investigators learn more about the expression of their specific gene, they will have to make constant comparisons between the abnormal cell and appropriate normal cells. They will need to prove that BON cells use the same mechanism of triggering NT gene expression as are used by normal cells and that the promoter region of the BON NT gene is unaltered. This laboratory group will obviously do that.

A question I would like to raise relates to forskolin and serotonin used in their experiments. These agonists tend to be fairly pleomorphic in their effects on the cell, a fact alluded to by the other discussants. Perhaps Dr. Evers could comment on why these two agents were selected instead of perhaps other agonists; for example dibutyl cyclic AMP or phosphodiesterase inhibitors such as theophyllin—reagents that have a more direct effect on cyclic AMP.

Finally the authors have implied that because the rat NT-gene promoter contains a DNA sequence that matches seven of eight nucleotides known

to be present in the CRE octamer control region of other cAMP-responsive genes, the NT gene in BON cells is likely to be mediated by a cAMP pathway. Although this may prove to be correct, it ultimately will depend on the cloning and sequencing of the promoter region of the BON-NT gene for more direct comparison and for the needed studies of NT-gene expression.

DR. M. DAVID TILLSON, III (New York, New York): You made some remarks in your introduction that I would like to ask you a question about, because I wonder if perhaps the loop can be closed now on some questions that I was interested in several years ago on the mechanisms related to adaptation of the gut and regulation of intestinal mass. I wonder if neurotensin is detectable in serum, and if addition of neurotensin stimulates intestinal growth in normal mucosa, even when it is not previously atrophied by starvation, and whether or not it is elevated after small bowel resection, and perhaps what would be most interesting to me of all, whether neurotensin might be the mechanism that we observed in some experiments years ago on the effect of small intestinal resection stimulating carcinogenesis in the rat colon in response to dimethylhydrazine?

I realize your paper was on a different subject, but is there a physiologic role for this hormone, is it a candidate for the regulation of intestinal mass, and does it perhaps have implications in terms of preparing the colon as a fertile soil for carcinogens?

DR. STANLEY R. FRIESEN (Kansas City, Kansas): There have been several interesting papers this afternoon that have dealt with an increasing spectrum of physiologic and pharmacologic actions of humoral substances, such as growth factors and neuropeptides. There are newer neuropeptides being described that have neurotransmitter or neuroendocrine actions.

Also it is increasingly apparent that these regulatory and trophic peptides reside not only in mucosal endocrine cells, but also in neural cells, such as ganglion cells of the gut and pancreas. When neural cells become neoplastic, they may liberate neuropeptides in excess. Examples include carcinoid-appearing tumors of the duodenal submucosa or of the pancreas; or, as in this interesting paper, a pancreatic carcinoid cell line that secretes neurotensin.

Historically it was Masson who described neural hyperplasia associated with carcinoid tumors of the gut. And Langerhans, a little over a hundred years ago, described a third group of cells in the pancreas, the neural ganglion cells between the acinar and the islet cells. It may be that neural cells constitute the cells of origin of gastrinomas that secrete the neuropeptide gastrin.

DR. B. MARK EVERS (Closing discussion): Dr. Debas, you asked about neurotensin mRNA levels in correlation with actual neurotensin protein levels. We found similar increases in both the message for neurotensin and neurotensin protein levels in BON cells after forskolin stimulation.

You next asked about possible functions of the two neurotensin transcripts. These two mRNA species result from the use of two different polyadenylation addition signals, and the two mRNAs differ in the extent of their 3' untranslated regions. It is interesting that the abundance of these two transcripts is different, depending on the tissue sampled. For example the gut has predominantly the smaller of the two mRNAs, whereas the brain has nearly equal amounts of both transcripts. We cannot say, however, whether this has any particular significance.

Dr. Andersen, you asked whether serotonin (5-HT) decreases neurotensin release. Ishizuka and colleagues, from our laboratory, have shown that 5-HT produces a dose-dependent decrease of cyclic AMP levels in BON cells, and, in addition, 5-HT decreases neurotensin release.

You also asked whether 5-HT may suppress endogenous neurotensin release given the fact that BON cells produce 5-HT. We suspect that there is some degree of basal suppression of neurotensin. In preliminary studies we have used a 5-HT receptor antagonist, LM-21009, which blocks 5-HT_{1A/1B} receptors. When LM-21009 is administered to BON cells, neurotensin release is increased. We are currently evaluating whether this increase in neurotensin release is correlated with an increase in neurotensin gene expression.

We did not measure cyclic AMP levels over a time course after administering forskolin. We postulate that the rapid changes in neurotensin mRNA levels are secondary to a relatively short half-life of the neurotensin transcript.

Dr. Niederhuber, we are currently evaluating the effects of other agents that increase intracellular cyclic AMP levels on neurotensin expression in BON. We used forskolin initially because it can directly activate adenylate cyclase and is quite stable.

Dr. Tilson, we have preliminary findings to suggest that neurotensin may play a role in the adaptive response of the remaining gut after small bowel resection. We have found that neurotensin mRNA levels are increased as early as 3 hours in the remaining small bowel after a 70% resection.

You also asked whether neurotensin could affect the growth of normal small bowel mucosa. It has been shown by several groups, including our own laboratory, that neurotensin can stimulate growth of normal small bowel mucosa and pancreas.

Dr. Friesen, you asked whether neurotensin could affect the growth of BON cells. Neurotensin has no effect on the growth of BON; however we have found that BON has no receptor for neurotensin.