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# Gabapentin-Induced Mitogenic Activity in Rat Pancreatic Acinar Cells

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**Gabapentin induces pancreatic acinar cell tumors in rats through unknown, yet apparently nongenotoxic mechanisms. The primary objective of this study was to determine whether gabapentin acts as a tumor promoter by stimulating acinar cell proliferation in rat pancreas. To this end, indices of pancreatic growth, including increased pancreatic weight, stimulation of acinar cell proliferation, and/or enhanced expression of immediate-early oncogenes were monitored in rats given gabapentin in the diet at 2 g/kg/day for up to 12 months. Rats fed raw soy flour (RSF), a known inducer of pancreatic acinar cell tumors through cholecystokinin-mediated mitogenic stimulation, were used throughout as positive controls. In addition, recent data suggests that gabapentin binds to the $\alpha_2\delta$ subunit of a voltage-gated, L-type calcium channel. Because signaling pathways for proliferative processes in pancreatic acinar cells involve intracellular calcium mobilization, the effects of gabapentin on intracellular calcium mobilization ($[Ca^{2+}]_i$) and $^3$H-thymidine incorporation were investigated in pancreatic acinar cells isolated from normal rat pancreas and in the AR42J rat pancreatic tumor cell line. As indicated by BrdU labeling indices, acinar cell proliferation increased 3-fold by Day 3 of RSF treatment and remained slightly greater than controls throughout the experiment. Pancreatic weights of RSF-fed rats were 32 to 56% greater than controls throughout the experiment. In contrast, gabapentin had no effect on pancreatic weight or acinar cell labeling index, and therefore had no apparent effect on pancreatic growth. In isolated pancreatic acinar cells, however, gabapentin induced mobilization of intracellular calcium and caused a slight increase in $^3$H-thymidine incorporation. The data suggest that gabapentin may possess low level mitogenic activity, which is not easily detectable in *in vivo* assays.**

*Key Words:* **acinar cell tumors; calcium mobilization; cholecystokinin (CCK).**

The anticonvulsant gabapentin induces pancreatic acinar cell tumors in male rats (Sigler *et al*., 1995), but has no activity in bacterial or mammalian mutagenicity assays, *in vitro* chromosomal aberration assays, or *in vivo* micronucleus assays, and therefore it appears to act via nongenotoxic mechanisms. The gastrointestinal hormone cholecystokinin (CCK) is a potent trophic factor for normal pancreas and may also act as a promoter in pancreatic tumorigenesis (Douglas *et al*., 1989; Howatson and Carter, 1985). Stimulation of endogenous CCK levels by xenobiotics, such as the sulfonyl urea compound A8947, has been shown to result in pancreatic hypertrophy and hyperplasia (Obourn *et al*., 1997). Further, in an azaserine-induced rat pancreatic tumor model, enhanced expression of CCK-A receptors, as well as expression of CCK-B receptors not normally expressed in rat pancreas, have been demonstrated in preneoplastic and neoplastic pancreatic foci (Bell *et al*., 1992; Zhou *et al*., 1992). Hypothetically, these chemically induced changes in pancreatic acinar cell receptor populations may increase sensitivity to endogenous trophic hormones such as CCK or gastrin, leading to enhanced cellular proliferation and tumor promotion. In preliminary experiments, however, we were unable to discern any effects of chronic gabapentin treatment on plasma CCK levels or on expression of pancreatic CCK-receptor subpopulations (de la Iglesia *et al*., 1997).

The objective of this study was to evaluate the mitogenic potential of gabapentin toward pancreatic acinar cells and test the hypothesis that the drug may behave as a tumor promoter through increased cell proliferation. To this end, indices of pancreatic growth, including increased pancreatic weight, acinar and ductal cell proliferation, and enhanced expression of immediate-early oncogenes in pancreas were assessed in rats given gabapentin in the diet at levels that induce pancreatic neoplastic growth. In addition, autoradiographic data have show that the rat pancreas accumulates gabapentin transiently after single oral doses (Vollmer *et al*., 1986), although radioligand binding assays in normal pancreas did not reveal specific gabapentin binding sites (Suman-Chauhan *et al*., 1993; Gee *et al*., 1996). Recent data suggest that gabapentin binds to the $\alpha_2\delta$ subunit of an L-type calcium channel (Gee *et al*., 1996). Because intracellular calcium ($[Ca^{2+}]_i$) plays an integral role in signaling pathways for proliferative as well as secretory processes in pancreatic acinar cells (Duan and Williams, 1994; Tsunoda and Owyang, 1995), we also examined the effect of gabapentin on $[Ca^{2+}]_i$ mobilization and cell proliferation in isolated normal pancreatic acinar cells and in AR42J rat pancreatic acinar cells *in vitro*. These latter data suggest that gabapentin-induced pancreatic tumorigenesis in rats may yet

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be related to weak promotional effects manifested through enhanced pancreatic acinar cell proliferation.

## MATERIALS AND METHODS

### In Vivo *Studies*

***Experimental animals.*** Three hundred and fifty-four male, random-bred, albino Wistar [Crl:(WI)BR]® were assigned randomly to groups and treated as indicated below until used for experimental analyses. Rats were housed individually in stainless steel cages and maintained in climate controlled rooms with 12-h light and dark cycles. Briefly, a group of 118 rats was fed gabapentin in the diet at 2000 mg/kg/day. One hundred and eighteen positive control rats were fed diet, *ad libitum*, containing raw soy flour (RSF) and 118 negative-control rats received Purina Certified Chow® (5002) until the time of termination, typically between 8 A.M. and 10 A.M. Subgroups of rats from each group were removed periodically for various assays as described below and in the tables and figure legends.

***Pancreatic cell proliferation studies* in vivo.** Pancreatic acinar and duct cell proliferation was measured in control, RSF-fed, and gabapentin-treated rats after 3, 7, 14, 31, 91, and 274 days of treatment. Cell proliferation rates were determined as labeling indices by measuring the percentage of cell nuclei incorporating the thymidine analog, bromodeoxyuridine (BrdU) during S-phase of the cell cycle. To label cells entering S-phase, osmotic pumps (Alza, Model 2ML1) containing 50 mg/ml BrdU in 0.5 N $NaCO_3$ were implanted subcutaneously 4 days prior to scheduled sacrifice. Upon necropsy, pancreata were weighed and examined macroscopically. Representative samples of morphologically normal-appearing pancreatic tissue were collected and processed for histopathological assessment and for morphometric determination of BrdU labeling indices. Measurements of acinar and ductal cell densities were also obtained by morphometry.

### In Vitro *Studies*

***Immediate-early oncogene analyses.*** Individual samples of pancreas or pancreatic nodules were collected on Day 3 and during Weeks 4, 39, 52, and 94 and frozen quickly in liquid nitrogen. Frozen tissue was homogenized in 1.5 ml Ambion® lysis/denaturation buffer and homogenates centrifuged at 4°C for 15 min at 14,000 × *g*. The resulting supernatant was transferred to new tubes and stored at –20°C until use. [$^{32}$P]-labeled RNA probes were synthesized from pTRI-B-Actin-125-Rat, pTRI-c-fos/exon4-Mouse, pTRI-c-myc/exon2-Mouse, and pTRI-jun-A-Mouse Ambion® DNA templates using the Ambion Maxi-Script™ *in vitro* transcription kit. Probes were purified from unincorporated nucleotides with Boehringer-Mannheim G-25 Sephadex Quick Spin™ Columns. Approximately 45 $\mu$l of pancreatic lysate was combined with 70,000 cpm of each of the RNA probes and hybridized overnight at 37°C.

Non-hybridized RNA was digested using the Ambion Direct Protect™ Lysate Ribonuclease Protection Assay Kit. Samples of equal protein content were resolved electrophoretically at 250 volts for approximately 1.5 h on 5% acrylamide/8M urea gels using a 0.9 M Tris/0.9 M boric acid/20 mM EDTA buffer system. Gels were dried, exposed to film, and autoradiographs were assessed by densitometry. The ribonuclease protection assay allows for measurement of specific mRNA directly on supernatants from tissue homogenates of limited size, as was obtained from these pancreatic nodules. Because homogenates contain a significant amount of protein, optical determination of RNA or DNA concentration at 260/280 nm was impractical because of crossover of protein absorbance into the 260 nm wavelength range. Therefore, binding of RNA probes was normalized to protein rather than DNA or RNA. Protein content in pancreatic lysates was determined by the method of Bradford (1976), using bovine serum albumin as a standard. Optical density was normalized to protein and expressed as OD/$\mu$g protein.

***Pancreatic acinar cell cultures.*** AR42J rat pancreatic tumor cells obtained from American Type Tissue Culture were grown in standard tissue culture flasks in Dulbecco's modified Eagle's medium (DMEM) containing 10% fetal calf serum. Primary cultures of normal acinar cells were prepared from rat pancreas according to collagenase digestion methods described by Lu and Logsdon (1992). Normal acinar cells were plated overnight in collagen-coated dishes in Waymouth's medium containing 10% fetal calf serum. Cultures were maintained in a humidified atmosphere of 95% oxygen/5% $CO_2$ at 37°C.

**In vitro *calcium mobilization.*** Effects of gabapentin on calcium mobilization were evaluated by laser cytometry (ACAS, Meridian Instruments, Okemos, MI) in normal pancreatic acinar cells and in AR42J pancreatic tumor cells. Calcium flux was measured by using the acetoxymethyl ester form of the fluorescent calcium chelator Fluo-3 at excitation and emission wavelengths of 488 and 515 nm, respectively. Cells were plated into 24-well tissue culture dishes at a density of $2 \times 10^4$ cells/well. Cells were pretreated with the fluorescent $Ca^{2+}$ indicator at 5 mM for 30 min, rinsed once, and covered with phenol red-free DMEM and treated with CCK-8 and/or gabapentin, as described in the figure legends.

***Cholecystokinin receptor competitive binding assays.*** To test the hypothesis that gabapentin's effects on intracellular calcium mobilization could be mediated by interactions at CCK receptors, ligand competition binding assays were conducted on normal rat pancreatic membranes as described above using 35 pM $^{125}$I-BH-labeled CCK-8S in the presence of increasing concentrations of unlabeled CCK-8 or gabapentin ranging from $10^{-16}$ to $10^{-5}$M.

***Cell proliferation* in vitro.** Effects of gabapentin on proliferation of normal pancreatic acinar cells and AR42J rat pancreatic tumor cells was assessed by $^3$H-thymidine incorporation. Cells were plated at 60,000/cm$^2$ in 12-well tissue culture dishes and incubated overnight. Medium was replaced with treatment solutions containing 1 $\mu$Ci $^3$H-thymidine and increasing concentrations of gabapentin as indicated in the figure legends. Cells were incubated for 18 h, after which trichloroacetic acid-precipitable radioactivity was quantitated by scintillation spectroscopy.

***Statistical analyses.*** Treatment groups were compared using a one-way analysis of variance (ANOVA) with Student-Newman-Keuls *post-hoc* test, where appropriate. A $p$-value $< 0.05$ was selected prior to the study, for determining statistical significance.

## RESULTS

### In Vivo *Experiments*

*Pancreas weight.* Rats fed normal diet exhibited time-dependent increases in pancreas weight consistent with normal organ growth in young rats. Pancreas weights in gabapentin-treated rats were comparable to controls throughout the study. In contrast, pancreatic weights in RSF-fed rats were 46, 32, 35, 56, 56, and 43% greater than controls on Days 3, 7, 14, 31, 91, and 274, respectively (Fig. 1).

*Pancreatic acinar cell proliferation* in vivo. Acinar cell labeling indices for controls and gabapentin-treated rats were essentially constant over the first 14 days of the study and ranged from approximately 8 to 15% (Fig. 2). Thereafter, acinar cell labeling indices in these rats decreased with time to less than 1% by Day 274. Relative to controls, acinar cell labeling index in RSF-fed rats increased 3-fold to approximately 33% by Day 3 of treatment and decreased toward control levels by Day 7. In general, acinar cell labeling indices in morphologically normal pancreatic tissue from RSF-fed rats were typically greater than controls, particularly on Days 31, 91, and 274, although the differences were not statistically significant. Increased pancreatic weight in RSF-fed rats was accompanied by pancreatic acinar cell hypertrophy, which was

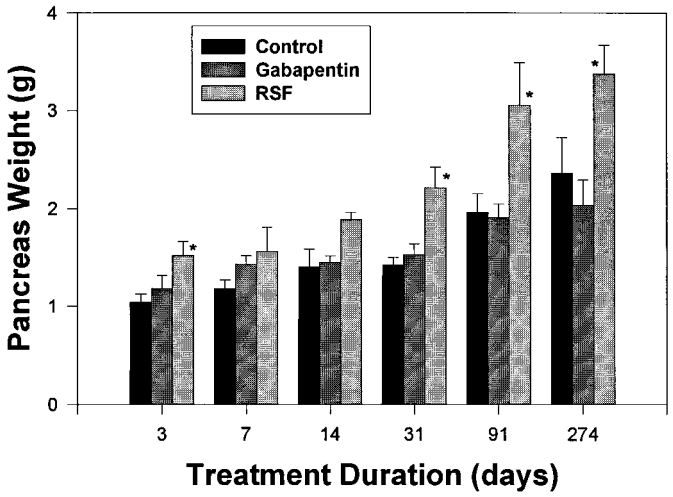


**FIG. 1.** Pancreas weight in RSF-fed or gabapentin-treated rats. Pancreata were weighed after various periods of treatment. histograms represent mean weight ± standard deviation for 5 rats per group. Asterisk signifies $p < 0.05$, Student-Newman Keuls test.

observed microscopically and reflected by decreases in acinar cell density (Table 1). Duct cell labeling indices appeared to show a similar time-dependent decrease, but the data were highly variable and there were no differences between treatment groups (Table 1).

*Emergence of pancreatic nodules.* Pancreatic nodules were generally detectable once they achieved a size of 2 mm. Nodules in the 2- to 3-mm size range were observed in one animal each in gabapentin- and RSF-fed groups at Day 31. At Day 91, additional, widely scattered nodules in this size range were observed, but in the RSF-fed group only. By Day 274, multiple 2- to 3-mm nodules were observed in gabapentin-treated as well as RSF-fed groups. Rats in the RSF-fed group also had nodules in the 3- to 8-mm range. Pancreatic nodules in untreated controls were rare, were not detected until Day 274, and were in the 2- to 3-mm size range. Nodules in the 2- to 5-mm range were considered foci of acinar cell hyperplasia based on histological characteristics. Typically, these nodules were characterized by well-differentiated exocrine cells forming normal acini and ducts. Nodules were usually devoid of islet cells. Pancreatic nodules in the 5- to 8-mm range were sharply demarcated from surrounding tissue, had well-differentiated acinar cells displaying a mild degree of nuclear pleomorphism, and exhibited crowding with variable cell size.

*Immediate-early oncogene expression.* Expression of immediate-early oncogenes c-*myc*, c-*fos*, or c-*jun* in grossly normal pancreas tissue collected from RSF-fed or gabapentin-treated rats after 3, 31, or 274 days of treatment was comparable to controls and there were no apparent time-dependent trends (data not shown).

Immediate-early oncogene expression was also evaluated in pancreatic nodules. Nodules from untreated controls and from gabapentin-treated rats were approximately 2 to 3 mm in size while nodules from RSF-fed rats ranged from 5 to 8 mm. Compared to normal tissue, oncogene expression was increased 2- to 4-fold in some pancreatic acinar cell nodules, whether harvested from control, RSF-fed, or gabapentin-treated rats (Figs. 3A–3C). Relative to normal tissue from untreated rats and relative to morphologically normal tissue neighboring the nodules, expression of c-*myc*, c-*fos*, and c-*jun* increased in these nodules from control and gabapentin-treated rats, while only c-*myc* and c-*jun* increased in nodular tissue from RSF-fed rats. Levels of c-*fos* in tissue from RSF-fed rats were comparable to morphologically normal pancreatic acinar tissue.

Increased oncogene expression was not observed in all pancreatic lesions, however. C-*myc* and c-*jun* increased in nodules harvested from RSF-fed rats after 52 weeks of treatment, but not in nodules obtained after 94 weeks. Conversely, c-*myc* and c-*jun* increased relative to morphologically normal tissue in pancreatic nodules taken from gabapentin-treated rats after 94 weeks, but not in nodules obtained after 52 weeks. In contrast to nodules from RSF-fed rats in which expression of c-*fos* was not increased, c-*fos* increased in nodules from gabapentin-treated rats after both 52 and 94 weeks of treatment. Relative to normal tissue, expression of all 3 immediate-early oncogenes increased in nodules obtained from control rats after 94 weeks.

## In Vitro *Experiments*

*Effects of gabapentin on calcium mobilization.* As indicated by a sharp increase in Fluo-3 fluorescence, CCK-8 at $10^{-8}$ M induced a rapid mobilization of calcium in AR42J cells,

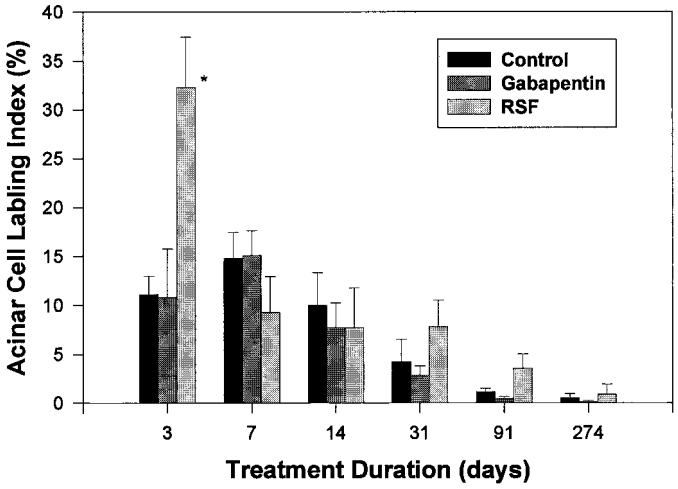


**FIG. 2.** Pancreatic acinar cell proliferation in RSF-fed and gabapentin-treated rats. BrdU labeling indices were determined morphometrically in RSF-fed and gabapentin-treated rats after various periods of treatment. Histograms represent mean labeling indices ± standard deviation in morphologically normal pancreatic tissue from 5 rats per group. Asterisk signifies $p < 0.05$, Student-Newman Keuls test.

**TABLE 1**
*In Vivo* Proliferation Indices in RSF-Fed and Gabapentin-Treated Rats

| Treatment | Treatment duration (days) | | | | | |
|---|---|---|---|---|---|---|
| | 3 | 7 | 14 | 31 | 91 | 274 |
| Acinar cell labeling index (%) | | | | | | |
| Control | 11.09 ± 1.89 | 14.19 ± 2.69 | 9.99 ± 3.34 | 4.22 ± 2.30 | 1.11 ± 0.37 | 0.49 ± 0.42 |
| Gabapentin | 10.81 ± 4.94 | 15.04 ± 2.56 | 7.73 ± 2.48 | 2.85 ± 0.95 | 0.45 ± 0.21 | 0.09 ± 0.14 |
| RSF | 32.24 ± 5.19* | 9.25 ± 3.67 | 7.71 ± 4.06 | 7.78 ± 2.72 | 3.54 ± 1.49 | 0.87 ± 1.06 |
| Duct cell labeling index (%) | | | | | | |
| Control | 5.27 ± 4.04 | 4.28 ± 3.67 | 8.22 ± 3.06 | 1.97 ± 1.61 | 1.27 ± 1.33 | 0.63 ± 0.87 |
| Gabapentin | 1.31 ± 1.35 | 8.52 ± 2.07 | 9.07 ± 2.11 | 3.60 ± 4.30 | 0.40 ± 0.55 | 0.09 ± 0.20 |
| RSF | 5.62 ± 4.91 | 5.46 ± 4.15 | 7.26 ± 4.05 | 4.83 ± 3.69 | 1.14 ± 1.22 | 4.95 ± 6.57 |
| Acinar cell density (cells/$\mu M^2$) | | | | | | |
| Control | 4.07 ± 0.43 | 3.91 ± 0.50 | 3.66 ± 0.31 | 3.29 ± 0.54 | 3.18 ± 0.18 | 3.79 ± 0.41 |
| Gabapentin | 4.40 ± 0.77 | 3.58 ± 074 | 3.43 ± 0.25 | 3.25 ± 0.42 | 3.34 ± 0.39 | 4.37 ± 0.56 |
| RSF | 3.52 ± 0.54 | 2.83 ± 0.31* | 2.33 ± 0.25* | 2.15 ± 0.22* | 2.12 ± 0.30* | 3.28 ± 0.53 |

*Note.* Mean ± standard deviation; $n = 5$.
*$p < 0.05$ relative to control, Student-Newman-Keuls test.

followed by an equally rapid return toward baseline levels (Fig. 4A). Gabapentin alone had no effect at $0.5 \times 10^{-7}$ M, but inhibited CCK-stimulated calcium transients. Subsequent addition of the calcium ionophore, ionomycin, at 2 $\mu$M confirmed the ability of gabapentin-treated cells to generate a calcium response (Fig. 4B). Sequential treatment of AR42J cells with CCK-8 and gabapentin demonstrated a rapid inhibition of CCK-8 induced calcium flux (Fig. 4C).

Similar to the response observed in the AR42J cells, normal pancreatic acinar cells rapidly mobilized calcium in response to stimulation with $10^{-8}$M CCK-8 (Fig. 5A), but the response in normal acinar cells appeared more robust than in the neoplastic cells. Like CCK-8, but in contrast to its effects on calcium mobilization in AR42J cells, gabapentin alone at $10^{-8}$ M stimulated rapid and relatively long-lasting increases in intracellular calcium and appeared not to block CCK-8-stimulated calcium mobilization in normal acinar cells (Fig. 5B). Continued responsiveness to CCK-8 stimulation could be demonstrated after exposure to gabapentin at $10^{-7}$ M (Fig. 5C).

*CCK receptor competition assays.* To determine whether gabapentin's inhibitory effects on AR42J cells and stimulatory effects in normal acinar cells were related to CCK receptor activity, competitive receptor binding studies were conducted

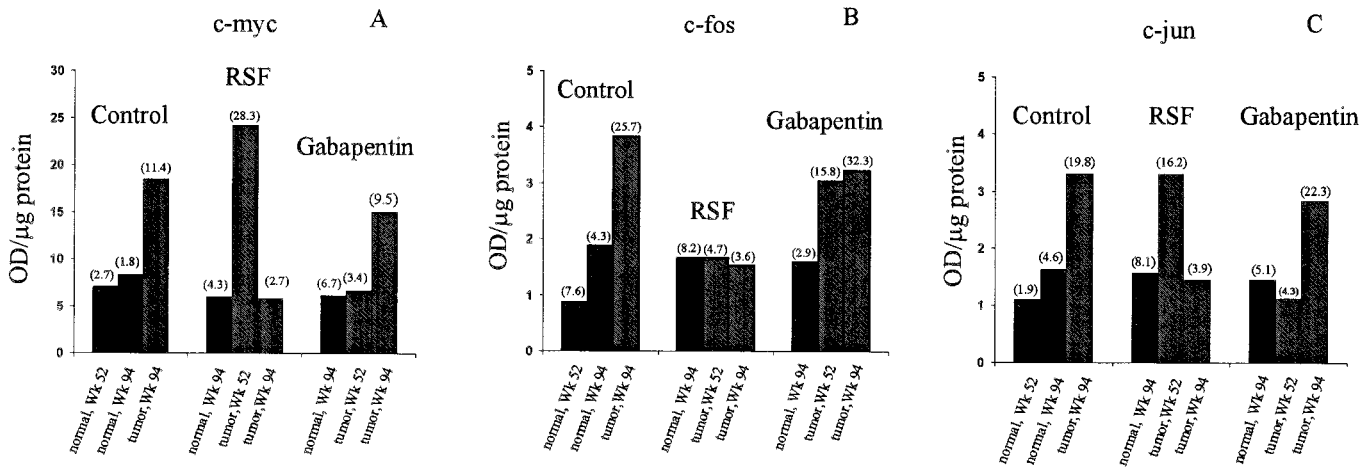


**FIG. 3.** Expression of immediate-early oncogenes in normal and nodular pancreatic tissue from RSF-fed and gabapentin-treated rats. Individual pancreatic nodules and morphologically normal tissue was collected and analyzed after 52 or 94 weeks of treatment. Immediate-early oncogenes were detected using an RNase protection assay and quantitated by densitometry. Histograms represent the average of 2 assays, with relative standard deviation as a percent of the mean shown in parentheses. The pancreatic nodules analyzed ranged in size from 2 to 3 mm for untreated control and gabapentin-treated rats and 5 to 8 mm for RSF-fed, respectively.

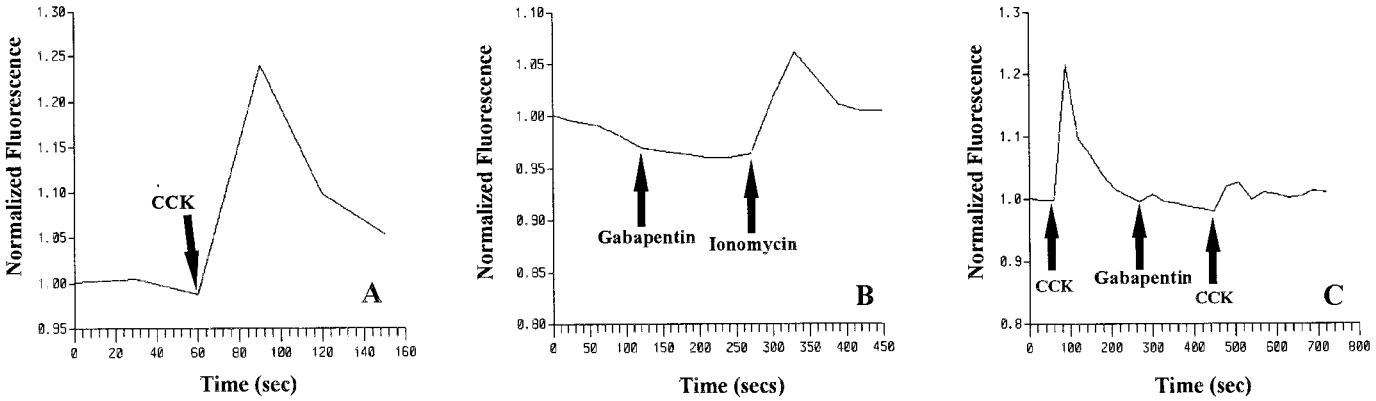


**FIG. 4.** Effects of gabapentin on calcium mobilization in AR42J cells. Fluo-3 labeled AR42J cells were treated with gabapentin alone or sequentially with gabapentin and CCK-8. Curves represent cumulative fluorescence for microscopic fields containing 10–15 cells.

in membranes isolated from normal rat pancreas using 35 pM $^{125}$I-BH-CCK-8S in the presence of increasing concentrations of unlabeled gabapentin (Fig. 6). Binding of CCK-8S was unaffected by gabapentin at concentrations as great as 1 $\mu$M, indicating that gabapentin does not bind with affinity to the CCK receptor, and thus is likely to act at a different site to modulate calcium mobilization.

*Pancreatic acinar and AR42J tumor cell proliferation* in vitro. Dose-related increases in $^{3}$H-thymidine incorporation into normal pancreatic acinar cells ranging from 240% to 320% were observed after 18-h incubations at gabapentin concentrations from 1 to 100 $\mu$g/ml, with statistically significant differences from control at 1 and 10 $\mu$g/ml (Fig. 7). Mean values for $^{3}$H-thymidine incorporation also appeared increased at 100 $\mu$g/ml, but there was great variability in the data and differences from control were not statistically significant. Thymidine incorporation into normal acinar cells decreased below control levels at gabapentin concentrations of 1- and 10-mg/ml, most likely due to cytoxicity at these extremely high doses. In contrast, gabapentin had no effects on proliferation of AR42J cell proliferation, in which $^{3}$H-thymidine incorporation was comparable to controls at doses ranging from 0.01- to 0.1-mg/ml. Similar to control acinar cells, thymidine incorporation in AR42J cells decreased below control levels at gabapentin concentrations of 1 mg/ml and above.

## DISCUSSION

In two-year carcinogenicity studies, gabapentin at 2000 mg/kg/day caused a statistically significant increase in acinar cell tumors in male Wistar rats, but not in female rats or B6C3F1 mice (Sigler *et al.*, 1995). Based on cytological features, a number of these neoplasia were categorized as acinar cell carcinomas, but the tumors did not metastasize. Onset and latency for gabapentin-induced tumors were the same as those in concurrent controls, and the tumors had no effect on survival or morbidity. Common exocrine pancreatic tumors in Wistar rats and in humans differ significantly based on the pathobiological characteristics. Rats develop acinar cell tumors (Rao, 1987), whereas humans manifest the highly malignant and

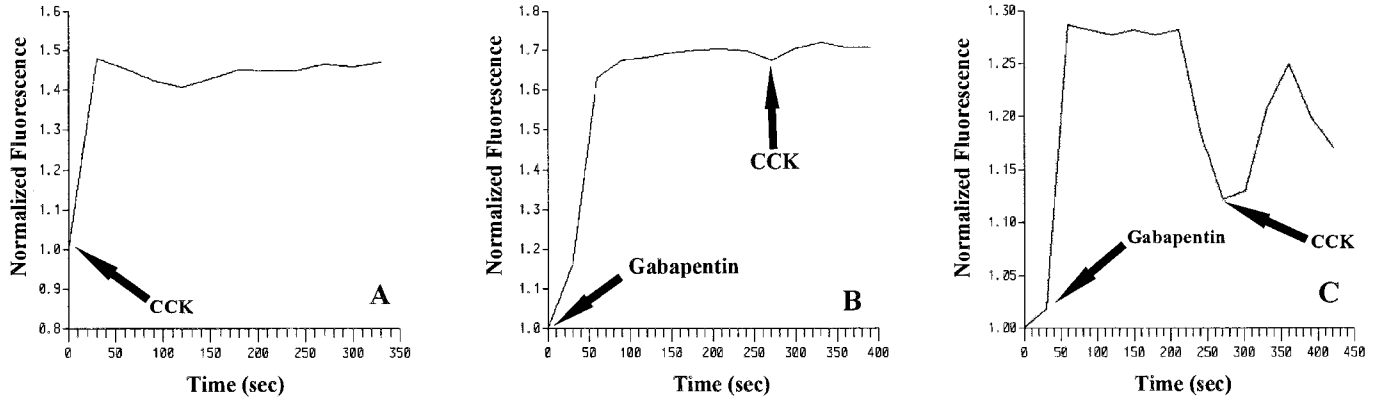


**FIG. 5.** Effects of gabapentin on calcium mobilization in normal pancreatic acinar cells. Acinar cells isolated from normal pancreas were pre-labeled with Fluo-3 and treated with gabapentin alone or sequentially with gabapentin and CCK-8. Curves represent cumulative fluorescence for microscopic fields containing 10–15 cells.

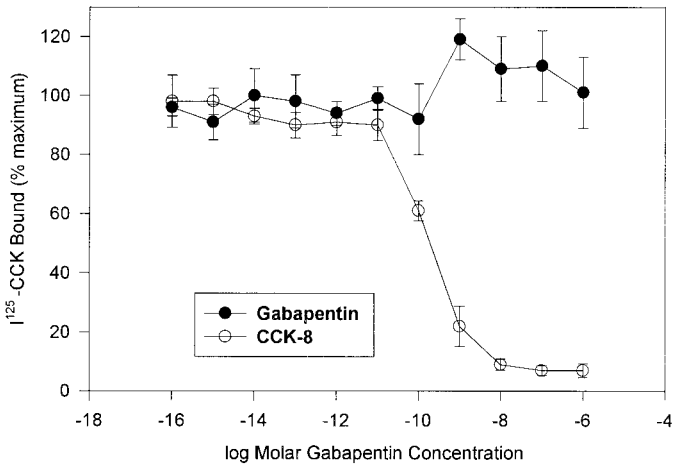


**FIG. 6.** CCK-receptor competition assays with gabapentin. To determine whether gabapentin's inhibition or stimulation of calcium mobilization in AR42J cells and normal acinar cells, respectively, may be related to interactions at the receptor level, receptor binding studies were conducted on normal rat pancreatic membranes using 35 pM $I^{125}$-Bolton Hunter-labeled CCK-8S in the presence of increasing concentrations of unlabeled gabapentin. Curves represent means ± standard deviation, where $n = 4$.

more aggressive duct cell carcinoma (Bockman, 1981; Maruchi *et al*., 1979). Based on the negative genotoxicity profile of gabapentin, pancreatic carcinogenesis in gabapentin-treated rats was considered to be an epigenetic phenomenon, and the weight of evidence suggests the potential for human risk to be very low.

The tumor biology of gabapentin-induced pancreatic acinar tumors appears similar to neoplasia occurring in rats spontaneously and in those given trypsin inhibitors or soya flour in the diet (Gumbmann *et al*., 1989; McGuinness *et al*., 1980). Cholecystokinin is a trophic factor for pancreas and has been implicated as a promoter in pancreatic carcinogenesis in several rodent models (Douglas *et al*., 1989; Howatson and Carter, 1985). Feeding soybean trypsin inhibitor contained in raw soy flour disrupts the trypsin-dependent negative feedback mechanism normally controlling CCK release and results in augmented CCK levels and pancreatic growth (Goke *et al*., 1986). Prolonged elevations in CCK caused by chronic trypsin inhibition in rats fed raw soya flour induces pancreatic acinar cell hyperplasia, adenomas, and carcinomas (Herrington *et al*, 1994). CCK has also been shown to enhance the development and shorten the induction time of preneoplastic acinar lesions in azaserine-treated rats (Povoski *et al*., 1993). Further, enhanced expression of CCK-A receptors or novel emergence of CCK-B/gastrin receptors could render neoplastic tissue more sensitive to stimulation by endogenous CCK or abnormally responsive to gastrin stimulation as described in the azaserine model of pancreatic carcinogenesis (Bell *et al*., 1992; Zhou *et al*., 1992). In separate, preliminary studies, we were unable to discern changes in CCK concentrations or in CCK receptor populations in pancreas of gabapentin-treated rats (de la Iglesia *et al*., 1997), although assay methodology for both may be problematic (Rehfeld, 1998).

Acinar cell proliferation and pancreatic weight were increased in positive control RSF-fed rats. These observations are consistent with previous reports and have been attributed to hormonal stimulation resulting from loss of CCK feedback inhibition (Goke *et al*., 1986). Had a similar trophic influence been present in gabapentin-treated rats, increases in acinar cell labeling and pancreatic weight should have been manifest in these rats as well.

Enhanced expression of immediate-early oncogenes, indicators of mitogenic activity, was not detected in normal pancreas tissue from a study of RSF-fed rats. Although tissues were evaluated as early as Day 3 of treatment, expression of these oncogenes is typically transient (Lu and Logsdon, 1992) and increases may have gone undetected. Expression of these oncogenes was increased in pancreatic acinar cell nodules occurring spontaneously in controls and in RSF-fed or gabapentin-treated rats, however. Increases in c-*myc* expression were reported in neoplastic tissue in the azaserine model (Silverman *et al*., 1990). Unfortunately, we could not discern the time course of oncogene expression relative to development of preneoplastic or neoplastic changes. C-*myc* and c-*jun* were increased in nodules taken from gabapentin-treated rats at

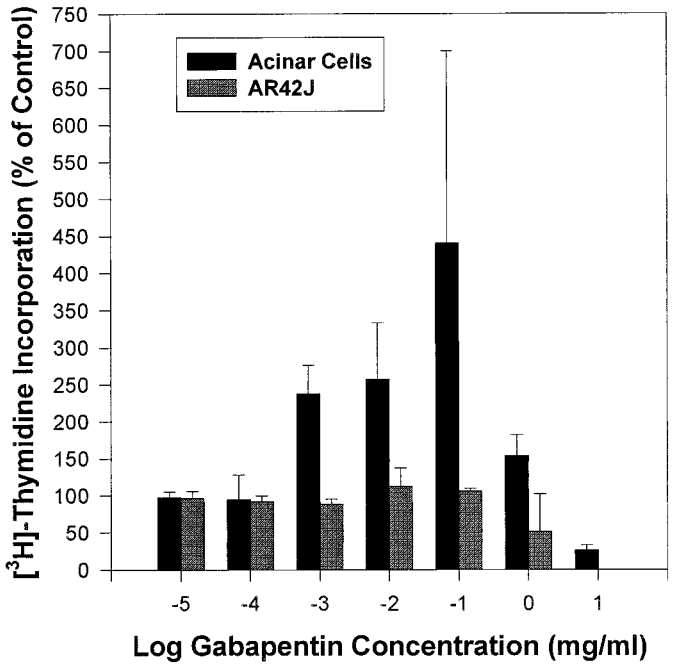


**FIG. 7.** Effect of gabapentin on normal acinar and AR42J tumor cell proliferation. Cell proliferation was evaluated using tritiated thymidine incorporation. Cells were plated at 60,000/$cm^2$ in 12-well tissue culture dishes and incubated overnight. Medium was replaced with treatment media containing 1 $\mu$Ci $^3$H-thymidine and increasing concentrations of gabapentin as indicated. Cells were incubated for 18 h and trichloroacetic acid-precipitable radioactivity was quantitated by scintillation spectrometry. $*p < 0.05$ where $n = 3$, Student-Newman-Keuls test.

Week 94, but not at Week 52, indicating that long-term treatment is needed and that the critical period of development lies between 1 and 1.5 years. Based on these cumulative data, gabapentin had no mitogenic effects demonstrable *in vivo*. Further, the data suggest that CCK does not play a significant role in gabapentin-induced pancreatic carcinogenesis.

Autoradiographic studies have shown that gabapentin accumulates transiently in pancreas following oral administration (Vollmer *et al*., 1986), but radioligand binding assays have not detected significant specific gabapentin binding sites in normal rat pancreas (Gee *et al*., 1996; Suman-Chauhan *et al*., 1993). Despite this absence of specific gabapentin binding sites in normal pancreatic tissue, it appears from our laser cytometric data that gabapentin alters normal pancreatic acinar cell calcium homeostasis. Further, the data suggest fundamental differences in the profile of calcium responses between normal pancreatic acinar cells and AR42J pancreatic tumor cells. Specifically, gabapentin increases intracellular calcium levels in normal pancreatic acinar cells and has no apparent effect on CCK-stimulated intracellular calcium mobilization. In contrast, in the AR42J rat pancreatic-tumor cell line, gabapentin alone has no apparent effect on calcium levels, yet inhibits CCK-stimulated intracellular calcium mobilization.

Recent data indicates that gabapentin binds to the $\alpha_2\delta$ subunit of a voltage-gated calcium channel, which is present in brain and skeletal muscle, but is not expressed in normal rat pancreas (Gee *et al*., 1996). The presence of L-type calcium channels has been demonstrated in the AR42J rat pancreatic tumor cell line, however (Christophe, 1994). It is conceivable that gabapentin might confer some growth advantage to acinar cells abnormally expressing L-type calcium channels through spontaneous mutation, but the presence of these binding sites in pancreatic acinar cell tumors from gabapentin-treated rats remains to be demonstrated. In the pancreas, calcium is a second messenger in the signaling cascade mediating acinar cell responses to trophic hormones, such as CCK (Louie, 1994). For example, the activated CCK receptor couples to G proteins and stimulates phospholipase C to catalyze the breakdown of phosphatidylinositol 4,5-bisphosphate to inositol 1,4,5-triphosphate ($IP_3$) and diacylglycerol (DAG). $IP_3$ stimulates the release of $[Ca^{2+}]_i$, while DAG activates protein kinase C in the plasma membrane. These latter events may act synergistically due to mutual molecular targets for $Ca^{2+}$-calmodulin-dependent phosphorylation as well as phosphorylation by protein kinase C. Additional events in the acinar cell proliferative response include stimulation of tyrosine kinase, activation of mitogen-activated protein kinase activity, and expression of immediate-early response genes such as c-*myc*, c-*fos*, and c-*jun* (Lu and Logsdon, 1992). Although we have demonstrated that gabapentin does not interact with CCK receptors, its ability to increase intracellular calcium suggests that gabapentin may activate postreceptor downstream effectors and trigger proliferative signaling pathways. Using incorporation of $^3$H-thymidine as an indicator of S-phase activity and cell proliferation, the *in vitro* data support the notion that gabapentin may stimulate DNA synthesis in normal pancreatic acinar cells. Concentrations at which gabapentin stimulated $^3$H-thymidine incorporation in normal acinar cells are comparable to the plasma concentrations of approximately 110 $\mu$g/ml, associated with increased pancreatic acinar cell tumors in rats (Sigler *et al*., 1995). Acting through this mitogenic pathway, gabapentin may behave as a weak tumor promoter, as has been established for CCK (Douglas *et al*., 1989; Howatson and Carter, 1985).

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