

# Altered Protein Kinase C Activity in Biopsies of Human Colonic Adenomas and Carcinomas<sup>1</sup>

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## ABSTRACT

Protein kinase C (PK-C) seems to be involved in the regulation of growth and differentiation of normal epithelial cells. Colonic adenomas and carcinomas show increased proliferation and decreased differentiation. We investigated the activity and subcellular distribution of PK-C in biopsies of normal, neoplastic, and malignant colonic epithelium to evaluate alterations in enzyme activity. In the control group ( $n = 7$ ), the activity of PK-C was highest in the distal ileum (597 pmol/min/mg protein) and declined to the lowest amounts in rectal mucosa (225 pmol/min/mg protein).

In patients with colonic adenomas ( $n = 16$ ), total PK-C activity was significantly reduced as compared to adjacent mucosa (146 versus 336 pmol/min/mg protein,  $P < 0.05$ ) and to values determined in the control group (372 pmol/min/mg protein,  $P < 0.01$ ). The reduction of total PK-C activity in the adenoma group was even more evident in intraindividual comparison to paired adjacent mucosa (41.8% of adjacent mucosa,  $P < 0.001$ ). Specific activity of membrane-associated PK-C was equally decreased in colonic adenomas (36.3 pmol/min/mg protein) when compared to adjacent mucosa (102 pmol/min/mg protein,  $P < 0.05$ ) or to the control group (107 pmol/min/mg protein).

In patients with colonic carcinomas ( $n = 10$ ), the amount of total PK-C activity was also decreased (198 pmol/min/mg protein) when compared to adjacent mucosa or to the control group ( $P < 0.05$ ). In addition, the amount of membrane-associated PK-C activity (89.1 pmol/min/mg protein) was significantly reduced in carcinomas when compared to adjacent mucosa ( $P < 0.05$ ).

The ratio of membrane-associated/total PK-C was not altered in adenomas, while in patients bearing carcinomas the relative fraction of membrane-associated PK-C activity was increased in samples from carcinomas and equally from adjacent colonic mucosa (45.0 and 44.6 versus 28.9%,  $P < 0.05$ ) when compared to controls.

These results indicate that alterations within the protein kinase C pathway occur as early events in the adenoma-carcinoma sequence of intestinal mucosa, suggesting an important role of PK-C in epithelial differentiation and growth.

## INTRODUCTION

The development of colonic carcinomas is thought to occur as a multistep process involving hyperproliferative adenomas as precancerogenic lesions (1). Thus, the analysis of biochemical pathways in patients with adenomas and carcinomas in relation to unaffected adjacent mucosa offers the possibility of investigating stages of colonic carcinogenesis within the same patient.

Various reports have correlated increased fecal amounts of bile and fatty acids or dietary habits to the occurrence of colonic adenomas and cancer (2-4). A highly correlated biochemical

marker is nevertheless still missing. Increased levels of oncogenes, gene deletions, altered growth factor activities, and growth factor receptor numbers have been related to advanced stages of neoplastic tissue (5-9). Furthermore, genetic predisposing factors may play a pivotal role in the susceptibility to colonic cancer, as demonstrated by pedigree studies (10-12) and by data concerning the general hyperproliferative activity within the entire intestinal mucosa of cancer patients (13, 14).

Stimulation of the the phosphoinositide system is supposed to be involved in cell growth and differentiation (15). Specific phospholipase C-dependent hydrolysis of the membrane lipid phosphatidylinositol-4,5-bisphosphate leads to the generation of the second messengers inositol trisphosphate and diacylglycerol (16). Inositol trisphosphate modulates intracellular calcium concentrations, while diacylglycerol activates a family of  $Ca^{2+}$ /phospholipid-dependent protein kinases (protein kinase C) (16, 17).

Activation of PK-C<sup>3</sup> has been shown to influence both induction and inhibition of DNA synthesis in different experimental systems (17-20). In addition, protein kinase C appears to promote differentiation of leukemic and epithelial cells, as demonstrated by an increase of membrane-associated PK-C in differentiating cells or by a lack of differentiation-like features in cells incubated with protein kinase C inhibitors (21-24).

The activation of PK-C seems to initiate a cascade of complex regulatory events, including regulation of transcription (17, 25). Prolonged activation of PK-C is supposed to down-regulate the enzyme and to inhibit various metabolic pathways (14, 17, 26). Thus, protein kinase C seems to both mediate stimulation of cell metabolism and additionally prevent the cell from uncontrolled activation. Therefore, alterations of the activity of PK-C may lead to disordered cellular metabolism and growth control (27, 28).

Involvement of PK-C in the induction of proliferation of intestinal epithelium has been demonstrated for bile- and fatty acid-stimulated DNA synthesis of isolated rat colonic mucosal cells (29, 30). Increased activity of ornithine decarboxylase was observed in colonic adenomas and carcinomas and may involve regulation by protein kinase C (31). Furthermore, phorbol ester treatment of primary cultures of human adenomas was demonstrated to cause an increase of DNA synthesis and secretion of plasminogen activator (32, 33).

Previous reports by Guillem *et al.* (34) have demonstrated decreased levels of protein kinase C activity in surgically resected colonic carcinomas as compared to adjacent normal mucosa. Since colonic adenomas may represent a hyperproliferative and premalignant state of the intestinal mucosa, we assessed whether the process of colonic carcinogenesis may be associated with early changes of PK-C activity already present in colonic adenomas. Total and membrane-bound PK-C activity

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<sup>3</sup> The abbreviations used are: PK-C, protein kinase C; EGTA, ethyleneglycol bis( $\beta$ -aminoethyl ether) $N,N,N',N'$ -tetraacetic acid; PS, phosphatidylserine; DAG, *sn*-1,2-diacylglycerol.

was determined in colonic mucosal tissue obtained during colonoscopy. Levels found in colonic adenomas and carcinomas were compared to levels determined intraindividually in adjacent mucosa or analyzed in relation to a control group.

**PATIENTS AND METHODS**

**Patients and Tissue Characteristics.** Biopsies from 33 patients undergoing diagnostic or therapeutic colonoscopy for colonic polyps and colonic carcinomas were investigated. The study was approved by the local ethics committee and written informed consent was obtained from all patients. Colonoscopy was performed with a flexible fiberoptic colonoscope (Olympus CLV) and biopsies were obtained with a miniforceps. Prior to colonoscopy the intestinal tube was cleaned by oral administration of 4–6 liters of irrigation fluid.

Adenomas ( $n = 16$ ) were localized in the sigmoid ( $n = 9$ , 3 of which were classified as tubulovillous), in the descending colon ( $n = 5$ , 2 tubulovillous), and in the transverse colon ( $n = 2$ , both tubular adenomas). The size of the adenomas was  $>1$  cm in 3 patients, while 13 had adenomas from 0.3–0.9 cm in diameter. Carcinomas were localized in the sigmoid colon (6 patients), the left side of the colon (2 patients), and the right side of the colon (2 patients). In patients with adenomas or carcinomas, biopsies were taken as paired samples from the adenoma/tumor and 10–20 cm away from the lesion from the normal-appearing mucosa.

Patients who underwent colonoscopy for diffuse abdominal pain ( $n = 3$ ), positive stool test for blood ( $n = 2$ ), or screening, because of diseases unrelated to the gastrointestinal tract ( $n = 2$ ), were used as controls. The groups of controls [ $n = 7$ ; age,  $59.6 \pm 16.6$  years (mean  $\pm$  SD); female/male, 4/3], patients with adenomas ( $n = 16$ ; age,  $62.9 \pm 9.3$  years; female/male, 8/8), and carcinomas ( $n = 10$ ; age,  $69.2 \pm 15.7$ ; female/male, 4/6) were not significantly different. The values of PK-C activity determined in the three colonic segments (cecum, transverse colon, and sigmoid colon) of these control patients were used to calculate representative total and membrane-associated PK-C activity in this group termed "control group."

Three to five samples (5–7 mg/biopsy) of normal intestinal mucosa, adenomas, and carcinomas were immediately frozen in dry ice and stored at  $-80^{\circ}\text{C}$  until analysis. Two corresponding biopsies were kept in formalin for histopathological examination, to control amounts of inflammatory, necrotic, or stromal tissue. Stromal contents were about 10% for normal mucosa, 12% for adenomas, and 20% for carcinomas. Further analysis to investigate a probable relation between the contents of diacylglycerol or PK-C activity and the amount of stromal tissue did not indicate a significant correlation (not shown).

Since proteolytic phospholipid/diacylglycerol-independent PK-C activity may be generated by freezing (35), control experiments were performed to compare PK-C activity of freshly analyzed tissue with data obtained from frozen tissue. In these experiments PK-C activity of freshly analyzed tissue was similar to values observed from frozen samples ( $n = 3$ ). Proteolytic phosphatidylserine/diacylglycerol-independent PK-C activity as measured by histone III-S phosphorylation in the absence of PS/DAG and the presence of  $\text{Ca}^{2+}$  and enzyme was always  $<2\%$  of PS/DAG/ $\text{Ca}^{2+}$ -dependent histone phosphorylation.

**Protein Kinase C Assays.** Protein kinase-C activity in cytosolic and membrane preparations was assayed by the incorporation of  $^{32}\text{P}$  into histone III-S (35). Briefly, tissue samples were homogenized in buffer A, containing 20 mM Tris-HCl, 10 mM EGTA, 2 mM EDTA, 0.1% mercaptoethanol, 0.25 M saccharose, 1 mM phenylmethylsulfonyl fluoride by sonication (3 times, 30 s, at  $4^{\circ}\text{C}$ ). The preparation was centrifuged at  $12,000 \times g$  for 15 min at  $4^{\circ}\text{C}$  (Mikro Rapid/K; Hettich, Tuttlingen, FRG) and the supernatant representing the cytosolic fraction was applied to DEAE-Sephacel (diethylaminoethyl cellulose 52, 100  $\mu\text{l}$  packed volume; Whatman) in Eppendorf tubes. DEAE-cellulose was previously equilibrated with buffer B, containing 20 mM Tris-HCl, 1 mM EDTA, and 0.1% mercaptoethanol. The membrane pellet was homogenized with Nonidet P-40 (1%) in buffer A at  $4^{\circ}\text{C}$ , sonicated, centrifuged at  $12,000 \times g$ , and applied to tubes with DEAE-cellulose,

as described for the preparation of cytosolic PK-C. To reduce the elution of other nonspecific kinases, DEAE-cellulose was washed twice with buffer B containing 25 mM NaCl. Cytosolic and particulate fractions were eluted with 120 mM NaCl in buffer B. Protein kinase-C activity was assayed by the addition of 10  $\mu\text{l}$  DEAE-eluate to a final volume of 40  $\mu\text{l}$  (containing 1.25 mg/ml histone III-S, 12.5 mM  $\text{MgCl}_2$ , 20 mM Tris-HCl, 0.1 mM  $\text{CaCl}_2$ , pH 7.5) and 65 mM  $[\gamma\text{-}^{32}\text{P}]\text{ATP}$  (200–400 cpm/pmol) and samples were incubated for 5 min at  $30^{\circ}\text{C}$ . Concentrations of free calcium were calculated with the computerized program "Calcon," kindly provided by Dr. J. S. Tash, Department of Cell Biology, Baylor College, Houston, TX. The assays were terminated by spotting 30  $\mu\text{l}$  of the reaction mix on Whatman P-81 phosphocellulose paper and the filters were immediately soaked into ice-cold trichloroacetic acid (20%). Unbound radioactivity was reduced by extensive washes in perchloric acid, water, and acetone, subsequently.

PS/DAG/ $\text{Ca}^{2+}$ -dependent histone III-S phosphorylation was performed in the presence of 10  $\mu\text{g}/\text{ml}$  PS, 1.0 mg/ml DAG, and 0.1 mM  $\text{Ca}^{2+}$ . The amount of  $^{32}\text{P}$  incorporation into histone in the absence of PS/DAG/ $\text{Ca}^{2+}$  and eluted enzyme and in the presence of EGTA (1.25 mM) was subtracted. Protein kinase activity is expressed as pmol/min/mg protein, determined in the cytosolic or particulate eluate, respectively. Protein was determined according to the method of Bradford (36), using bovine  $\gamma$ -globulin as standard. We did not calculate PK-C activity relative to tissue DNA contents since carcinomas frequently present hyperploid states of cellular DNA.

Fig. 1 shows a representative purification of PK-C from DEAE-cellulose by stepwise elution with NaCl (0–500 mM). PK-C activity eluted between 25 and 125 mM NaCl and was strictly dependent on the presence of PS and DAG.

To exclude that differences between investigated tissues may result from different sensitivities to PK-C activating compounds, assays with various concentrations of PS, DAG, and calcium were performed. Cytosolic PK-C activity determined from human colonic mucosa, adenomas, and carcinomas ( $n = 3$ , for each group) was all highly dependent on PS and DAG. Similar  $K_m$  and  $V_{max}$  values for the cofactors and substrates were obtained for the adenoma/carcinoma and control groups. Calcium alone did not stimulate  $^{32}\text{P}$ -histone phosphorylation.

**Data Analysis.** Data are reported as means  $\pm$  SEM. Statistical analysis was performed by Student's  $t$  test for paired samples and by the nonparametric Mann-Whitney  $U$  test.

**Drugs and Radiochemicals.** All reagents were of analytical grade and purchased from Merck (Darmstadt, FRG), unless indicated otherwise. Phenylmethylsulfonyl fluoride was from Serva (Heidelberg, FRG). Diethylaminoethyl cellulose 52 and Whatman P-81 phosphocellulose paper were from Whatman (Maidstone, Kent, United Kingdom).  $[\gamma\text{-}^{32}\text{P}]\text{ATP}$  was from Amersham Buchler (Braunschweig, FRG). Histone

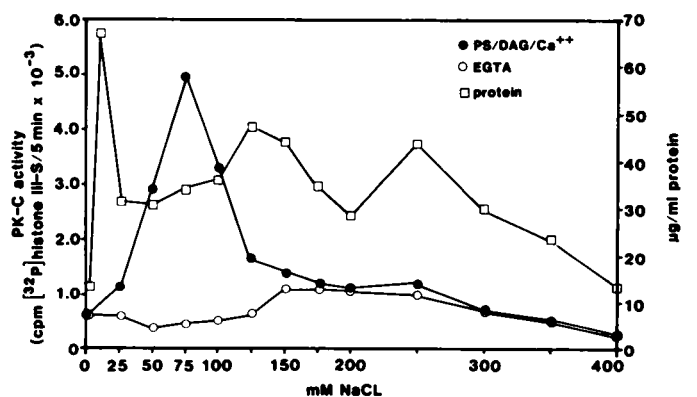


Fig. 1. Elution profile of soluble PK-C on DEAE-cellulose. DEAE-cellulose purification of soluble protein kinase C activity from biopsies derived from patient 5 (72 years old, tubular adenoma of the descending colon). The soluble fraction of PK-C was prepared as described in the "Patients and Methods" and applied to DEAE-cellulose and eluted stepwise with the indicated concentrations of NaCl (fraction volume, 0.5 ml). Fractions were assayed for PK-C activity in the presence of PS/DAG/ $\text{Ca}^{2+}$  or with EGTA (1 mM) alone. Protein concentrations were determined according to the method of Bradford (36). Results shown are from a single representative experiment, repeated twice.

III-S, diacylglycerol, and phosphatidylserine were from Sigma (Taufkirchen, FRG), and Nonidet P-40 was from LKB (Bromma, Sweden).

**RESULTS**

To investigate a possible involvement of altered protein kinase C activity in the development of colonic adenomas and colonic carcinomas, total PK-C and membrane-associated PK-C activity of 33 patients was determined. PK-C activity of patients with colonic adenomas ( $n = 16$ ) and those with colonic carcinoma ( $n = 10$ ) was compared to PK-C levels in adjacent mucosa and to a group of 7 control patients.

**PK-C Activity in Colonic Mucosa of Control Patients.** To obtain an estimate of enzyme activity in normal intestinal mucosa, PK-C was determined in various segments of the intestinal mucosa of 7 patients having a macroscopically and histopathologically normal mucosa. Samples from the distal ileum, cecum, transverse colon, sigmoid colon, and rectum were analyzed (Table 2). Highest PK-C levels were found in the distal ileum ( $597 \pm 200$  pmol/min/mg protein), while levels in the cecum ( $402 \pm 108$  pmol/min/mg protein), transverse colon ( $281 \pm 79$  pmol/min/mg protein), and sigmoid colon ( $434 \pm 134$  pmol/min/mg protein) declined to the lowest levels in the rectum ( $225 \pm 98$  pmol/min/mg protein). Values for membrane-associated PK-C activity were similar in all intestinal segments investigated (range, 25.1–37.6%, Table 1). Although the amounts of total PK-C activity varied within a wide range in these groups, the intraindividual levels of PK-C in the various intestinal segments were within narrow limits and showed a continuous decline from the distal ileum to the rectal mucosa ( $45 \pm 9.7\%$ , as compared to levels in the distal ileum,  $P < 0.002$ , Fig. 2). Total and membrane-associated PK-C activity was calculated from the colonic segments, cecum, transverse colon, and sigmoid colon (total,  $372 \pm 79$  pmol/mg/min protein; membrane-associated fraction,  $107 \pm 7.1$  pmol/min/mg protein) and was used to compare PK-C activity determined in colonic adenomas and carcinomas.

**Patients with Colonic Adenomas.** To investigate whether possible changes of PK-C activity may be related to the occurrence of hyperproliferative intestinal diseases, PK-C activities in 16 patients with tubular ( $n = 11$ ) and tubulovillous ( $n = 5$ ) adenomas with low or moderate degrees of atypia were analyzed. The total activity of PK-C was significantly lower in colonic adenomas as compared to adjacent mucosa ( $146$  versus  $336$  pmol/min/mg protein,  $P < 0.05$ ) and in relation to the control group ( $372$  pmol/min/mg protein,  $P < 0.05$ ). Determination of specific membrane-associated PK-C activity in adenomas ( $36.3$  pmol/min/mg protein) indicated a similar decrease when compared to adjacent mucosa ( $102$  pmol/min/mg protein,  $P < 0.05$ ) and to mucosa from control patients ( $P < 0.05$ , Table 2). The subcellular distribution of PK-C, as determined by the ratio

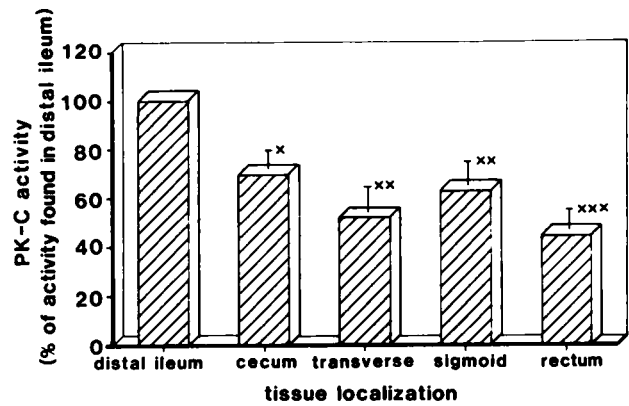


Fig. 2. Relative distribution of PK-C in various segments of intestinal mucosa. Intraindividual comparison of total PK-C activity determined in 3–5 biopsy specimens from various segments of intestinal mucosa from 7 healthy patients (control group). PK-C activity was determined as described in "Patients and Methods." Levels of PK-C activity determined in distal ileum ( $597 \pm 200$  pmol/min/mg protein) were taken as 100%. Absolute amounts of PK-C activity are given in Table 1. Columns, means; bars,  $\pm$ SD; x,  $P < 0.025$ ; xx,  $P < 0.01$ ; xxx,  $P < 0.002$ .

of membrane-associated/total PK-C activity was not significantly altered in adenomas (24.9%) or adjacent normal mucosa (30.6%) in comparison to values observed in the control group (28.9%, Table 2). As shown in Fig. 3, the decrease in PK-C activity was consistently observed in adenomas when compared to paired adjacent mucosa within each individual ( $41.4 \pm 7.9\%$ ,  $n = 16$ ,  $P < 0.001$ ). Hence, alterations of the protein kinase C pathway are associated with the occurrence of early neoplastic but premalignant stages of intestinal mucosa. Further analysis of the data in relation to the histopathological classification of the adenomas (tubular versus tubulovillous) did not indicate significant differences within either groups although total PK-C levels in tubulovillous adenomas ( $n = 5$ ) were slightly decreased, as compared to tubular adenomas ( $n = 9$ ) (not shown).

**Patients with Colonic Carcinomas.** In 10 patients with colonic carcinomas total PK-C activity was similarly decreased in carcinomatous tissue, as compared to the control group ( $198$  versus  $372$  pmol/min/mg protein,  $P < 0.05$ ) and to adjacent normal mucosa ( $336$  pmol/min/mg protein,  $P < 0.05$ , Table 2). The intraindividual comparison of PK-C activity from colonic carcinomas and adjacent mucosa indicated a decrease in 8 of 10 patients ( $65 \pm 9.4\%$ ; range, 20–95%), while 2 patients in this group had increased amounts of total PK-C activity ( $129 \pm 7.5\%$ ).

PK-C activity from carcinoma tissue derived from different colonic segments (sigmoid,  $n = 6$ ; right side,  $n = 2$ ; left side,  $n = 2$ ) was not correlated to the site of origin (not shown).

The analyses of the membrane-associated fraction of PK-C in carcinomas revealed decreased values of  $89.1$  pmol/min/mg protein in relation to amounts determined in adjacent mucosa ( $149$  pmol/min/mg protein,  $P < 0.05$ ; Table 2).

The ratio of membrane-associated/total PK-C activity was significantly increased in carcinomatous tissue and in adjacent mucosa of patients bearing carcinomas, as compared to control levels ( $45.0$  and  $44.6$  versus  $28.9\%$ ,  $P < 0.05$ ; Table 2). Since the increased association of PK-C with the membrane is thought to reflect the activated state of the enzyme, this might indicate a altered subcellular distribution of activated PK-C in colonic carcinomas and adjacent mucosa of patients with colonic cancer.

Table 1 Distribution of total PK-C activity and membrane-associated PK-C activity in various regions of intestinal mucosa of 7 healthy patients.

Localization	Total PK-C Activity (pmol/min/mg protein)		% membrane-associated PK-C activity (% total PS/DAG/Ca <sup>2+</sup> -dependent phosphorylation of histone III-S) Mean $\pm$ SEM
	Mean $\pm$ SEM	Range	
Distal ileum	597 $\pm$ 200	73–1194	37.6 $\pm$ 8.8
Cecum	402 $\pm$ 108	42– 677	30.6 $\pm$ 6.2
Transverse	281 $\pm$ 79	23– 528	30.9 $\pm$ 5.6
Sigmoid	434 $\pm$ 134	15– 824	25.1 $\pm$ 6.1
Rectum	225 $\pm$ 98	36– 652	27.8 $\pm$ 7.4

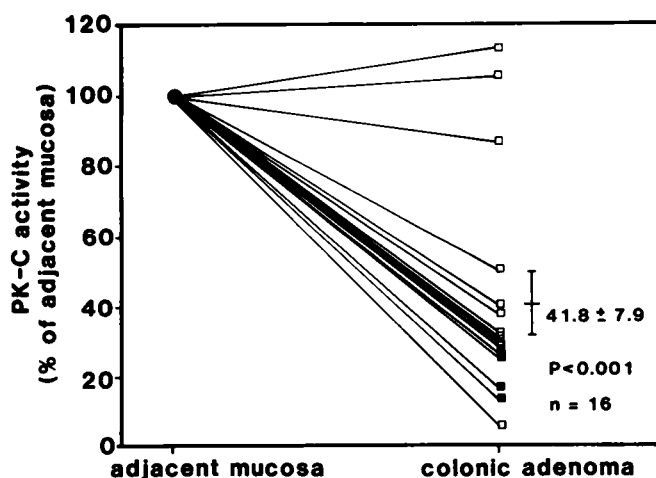
**Table 2** Total and membrane-associated PK-C activity in colonic adenomas compared to values determined in adjacent mucosa and to values found in the control group (mean  $\pm$  SEM)

Tissue derived from:	PK-C activity (pmol/min/mg protein)		
	Total	Membrane-associated	Ratio: membrane-associated vs. total (% PS/DAG/CA <sup>3+</sup> -dependent histone phosphorylation)
Control group (n = 7)	372 $\pm$ 79	107 $\pm$ 7.1	28.9
Adjacent mucosa (n = 16)	336 $\pm$ 87	102 $\pm$ 15	28.9
Colonic adenomas (n = 16)	146 $\pm$ 51 <sup>a</sup>	36.3 $\pm$ 6.6 <sup>a</sup>	30.6
Adjacent mucosa (n = 10)	336 $\pm$ 74	149 $\pm$ 23	44.6 <sup>c</sup>
Colonic carcinomas (n = 10)	198 $\pm$ 59 <sup>a</sup>	89.1 $\pm$ 6.9 <sup>b</sup>	45.0 <sup>c</sup>

<sup>a</sup> Significantly different ( $P < 0.05$ ) from values found in the control group and those obtained in adjacent mucosa.

<sup>b</sup> Significantly different ( $P < 0.05$ ) from values determined in adjacent mucosa.

<sup>c</sup> Significantly different ( $P < 0.05$ ) from values found in the control group.



**Fig. 3.** Comparison of PK-C activity of paired samples from colonic adenomas and adjacent mucosa. Intraindividual comparison of total PK-C activity determined in 3–5 biopsy specimens from colonic adenomas and adjacent mucosa (100%) of 16 patients. Total PK-C activity was determined as described in "Patients and Methods." Absolute amounts of determined total PK-C activity are given in Table 1. □, relative amounts of PK-C activity of tubular adenomas (n = 9); ■, levels of tubulovillous adenomas (n = 5); points, means; bar,  $\pm$ SD.

## DISCUSSION

In the present study we investigated whether changes occur in total and membrane-associated PK-C activity in colonic adenomas and carcinomas in humans. Neoplastic lesions were compared to normal mucosa from a control group and to adjacent normal mucosa of each patient. The data indicate a highly significant decrease of total PK-C activity in colonic adenomas, both in relation to normal mucosa of a control group and to intraindividual levels in paired normal colonic mucosa. Colonic carcinomas also contained significantly less total PK-C activity as compared to adjacent mucosa and to control patients. The ratio of membrane-associated/total activity of PK-C, which is supposed to represent the activated state of the enzyme, was not altered in normal mucosa of patients bearing adenomas or in the adenomas themselves. By contrast, colonic carcinomas and also normally appearing mucosa adjacent to carcinomas had an elevated relative fraction of membrane-associated PK-C activity, although specific membrane-associated PK-C activity was quantitatively decreased. The data are presented as specific PK-C activity per min and mg of protein. Similar data were obtained when the results were calculated for the amounts of tissue wet weight (not shown).

These data support previous reports of Guillem *et al.* (34) who investigated PK-C activity in surgically resected colonic

tumors. The authors similarly found a decrease of cytosolic and membrane-associated PK-C activity in colonic carcinomas when compared to adjacent mucosa. Based on the finding that carcinoma samples containing considerable amounts of benign adenomatous tissue had high levels of membrane-associated PK-C activity, the authors speculated that alteration of PK-C metabolism might already be present in premalignant colonic adenomas. The present study correlates PK-C activity of samples derived from colonic adenomas and carcinomas to adjacent mucosa and provides information about PK-C activity in intestinal mucosa of a normal control group. Our data demonstrate that both total and membrane-associated PK-C activity are significantly decreased in samples from colonic adenomas and carcinomas. In patients bearing carcinomas we observed a relative increase in the ratio of membrane-associated/total PK-C activity, probably indicating a permanently activated state of PK-C in patients susceptible to the development of colonic cancer.

The significance of quantitative and subcellular changes of PK-C activity in human colonic tissue determined under *ex vivo* conditions is not clear at present. However, the early decrease of PK-C activity in premalignant colonic adenomas and the altered activity and impaired subcellular distribution of PK-C in colonic carcinomas might suggest an important role of PK-C in the regulation of intestinal growth and differentiation.

Total protein kinase C activity of intestinal mucosa was generally higher in the distal ileum compared to various segments of the colon. This finding might be of interest since the development of carcinomas of the small intestine is relatively rare. However, the observed distribution might also be related to different metabolic activities of the mucosa in various segments of the intestine.

The role of protein kinase C in normal and neoplastic human colonic mucosa is not well established. Craven *et al.* (29, 30) reported an increased synthesis of DNA upon treatment with bile acids or fatty acids, which also caused an initial translocation of PK-C to the membrane with a subsequent down-regulation of the enzyme. Friedman *et al.* (32, 33) observed an increased labeling index, suggesting a proliferative response in primary cultures of human colonic tubular adenomas upon treatment with phorbol esters. Cultures of tubulovillous adenomas and carcinomas showed no growth response but reacted with secretion of plasminogen activator to phorbol esters. However, activation of PK-C was associated with differentiation and growth arrest in fibroblasts (20), keratinocytes (24), and several bone marrow-derived tumor cell lines (21, 23, 37). Thus, there is evidence for a role of PK-C in regulating both growth and

differentiation in epithelial cells, depending on the state of differentiation or transformation of the cell.

In our study changes of total protein kinase C activity were already evident within the adenoma group, as compared to adjacent mucosa and the control group. At present no biochemical marker is available to distinguish colonic adenomas from adjacent intestinal mucosa, despite the hyperproliferative behavior of adenomas. Various alterations have been observed in large adenomas and carcinomas of the colon such as *ras* oncogene mutations, genetic deletions, and differences in growth factor secretion and receptor capacity (5–9). Since these changes were mainly associated with colonic carcinomas and less frequently with adenomas, they appear to occur during tumor progression. The loss of PK-C activity in adenomas therefore may indicate that investigations of the PK-C pathway should be promising to detect further early alterations in the colonic adenoma-carcinoma sequence.

The specific activity of total and membrane-associated PK-C was decreased in colonic adenomas and carcinomas. It is not well established at present whether down-regulation of PK-C might lead to an inactivation of PK-C or involves a persistent functionally active fraction of PK-C which escapes detection. Very recent data from our laboratory have shown a decrease of diacylglycerol levels in colonic adenomas and carcinomas (38). Since activation of PK-C mainly depends on diacylglycerol, these results probably argue against an increased phosphoinositide metabolism generating diacylglycerol and thereby activating PK-C in colonic adenomas and carcinomas. In addition, analysis of phospholipase C activity, the key enzyme for phosphoinositide hydrolysis, indicated no difference in enzyme activity in colonic adenomas and paired adjacent mucosa.<sup>4</sup> However, changes in PK-C activity may be induced by mechanisms unrelated to phosphoinositide breakdown, since various fatty acids, increased concentrations of calcium, several phospholipids, and arachidonic acid were reported to activate PK-C (26, 39).

A variety of carcinogenic factors may be involved in the regulation of PK-C activity in colonic mucosa, either present in the luminal contents or related to genetic alterations. Increased fecal amounts of bile acids and fatty acid and dietary habits, such as high fat or protein intake and low fiber consumption, were correlated to the occurrence of colonic adenomas and carcinomas (2–4). Some of these factors, such as bile acids, are known to activate PK-C and might explain the relative increased ratio of membrane-associated/total PK-C activity, as determined in the group with colonic carcinomas. The finding that a relatively increased membrane-associated fraction of PK-C was already present in adjacent mucosa of tumor patients (Table 2) is compatible with the existence of hereditary cofactors involved in colonic carcinogenesis (11–14). Recently, the gene probably associated with familial adenomatous polyposis and the development of colonic carcinomas was localized on chromosome 5 (40, 41). The relative increase in the ratio of membrane-associated PK-C activity in colonic carcinomas might additionally be due to cocarcinogenic factors activating phosphoinositide metabolism, such as *ras* oncogenes (42), which were frequently found in human adenomas and carcinomas (5, 6, 9).

Nishizuka (26) proposed a role of negative feedback control on cell metabolism mediated by PK-C. Therefore, the decreased levels of PK-C in colonic adenomas and carcinomas could

promote uncontrolled growth of colonic mucosa, which might support the progression to a neoplastic or malignant phenotype. The association of increased levels of PK-C and differentiation was recently confirmed by Makowske *et al.* (37), who reported an increase of mRNA of PK-C in HL-60 cells upon differentiation with dimethyl sulfoxide and retinoic acid. Recent data from Guillem *et al.* (43) demonstrated increased mRNA levels of a specific protein kinase C-induced gene (phorb) in colonic carcinomas compared to adjacent mucosa. The expression of phorb mRNA was additionally positively correlated to tumor progression. These results might indicate a permanent activation of protein kinase C during colonic carcinogenesis leading to down-regulation of the enzyme.

In summary our data indicate that a decrease of total protein kinase C activity is related to the occurrence of hyperproliferative and premalignant diseases in human colonic mucosa. The decrease of total protein kinase C activity either might be due to down-regulation of PK-C as a result of hyperactivation or could indicate hereditary alterations of the pathways regulating the activity of PK-C. Thus, alterations of the PK-C pathway seem to occur as an early event within the human adenoma-carcinoma sequence.

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