Colorectal cancer metastases affect the biochemical characteristics of the human liver \(\beta\)-adrenoceptor-G-protein-adenylate cyclase system

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Abstract

The sympathetic-catecholamine system is involved in the regulation of hepatic metabolic pathways mainly through cAMP-linked \(\beta_2\)-adrenoceptors (\(\beta_2\)-ARs) in humans and to a lesser extent through cAMP-independent mechanisms, but no information is available about the possible biochemical changes of \(\beta_2\)-ARs and their signalling pathways in human colorectal cancer (CRC) and colorectal cancer hepatic metastases (CRCHM). Changes in density and distribution of \(\beta\)-ARs as well as in post-receptor signalling components were studied in membranes of human liver with CRCHM, and for comparison, in membranes of nonadjacent, non-metastatic human liver (NA-NM) obtained from 13 patients, using binding and competition binding studies. Studies were also carried out using normal and cancerous human colon tissues. In CRCHM, the density of \(\beta\)-ARs (\(B_{\text{max}}\)) was significantly reduced, compared to NA-NM liver tissues (40.09 ± 2.83 vs. 23.09 ± 3.24 fmol/mg protein; \(P<0.001\)). A similar decrease in the \(\beta\)-AR density was observed in the colon with primary colorectal cancer compared to healthy colon (37.6 ± 2.2 vs. 23.8 ± 3.5 fmol/mg protein), whereas the affinity of ICYP binding to the receptor remained unaffected. Desensitized \(\beta\)-ARs were uncoupled from stimulatory G-protein (G\(_s\)), as total density of \(\beta\)-adrenoceptors in the high affinity state was significantly reduced. Concomitantly, CRCHM elicited decrease in the catalytic adenylate cyclase (AC) activity (cAMP formation) in response to isoproterenol plus GTP or forskolin or NaF. In NA-NM and CRCHM liver, the inhibition–concentration curves of ICI 118.551 showed the presence of a homogeneous population of the \(\beta_2\)-AR subtypes. Neither the binding patterns nor the inhibition constant (\(K_i\)) of ICI 118.551 were altered in CRCHM. In CRCHM, the hepatic \(\beta\)-AR-G-protein(s)-AC signalling system was markedly impaired, thus, these changes may well influence \(\beta\)-AR-mediated functions in both organs.

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1. Introduction

Metastatic processes in the liver, in particular from colorectal cancer, are responsible for most cancer related deaths in the world [1,2]. The liver is the most common site of colorectal cancer metastasis and frequently the only affected organ. Up to 35% of patients with colorectal cancer will have hepatic metastases at time of surgery for the primary lesion, and further, 8% to 25% will develop metachronous hepatic metastases after primary resection [3]. In spite of extensive efforts over the last decades, only modest improvements have been accomplished in overall survival of patients with CRCHM through refinements of surgery, chemotherapy and ablation. Even with successful resection followed by chemotherapy, 5-year survival has been documented in only 30% to 40% of patients [4]. There is still lack of data that would contribute in elucidating the underlying mechanisms and identifying lethal processes caused by CRC and CRCHM.

The mechanisms have been viewed as being multifactorial with interrelated metabolic, neurogenic, cellular and inflammatory components. For example, there is evidence that metastatic cells involve a cascade of linked and sequential steps, including changes in the cytoskeleton, loss of adhesion, increased cellular motility and the release of proteolytic enzymes that degrade the basement membranes [5,6]. In
addition, it has been suggested as a common problem in patients with liver malignancies that there are alterations in carbohydrate metabolism in the liver. This presumably arises from enhanced glucose utilization by the tumor resulting in hypoglycaemia [7–9] as well as an increase in lipolysis [10] and protein catabolism [11], in which all are under the control of the autonomic nervous system with adrenergic receptors [12,13]. In defence against such severe metabolic alterations, especially hypoglycaemia, as a glucose-raising counter-regulatory mechanism, an increase in circulating catecholamine levels has been documented in various experimental studies in the liver involving sympathetic activation [14–16], but not in tumor patients. An important but poorly understood aspect of host responses to metastatic or primary tumor challenge and metabolic consequences is the interaction of the adrenergic fibers of the sympathetic nervous system and immune system that finally triggers severe phthisis in tumor patients [17–19]. Thus, it is still further contingent on assessing distinct pathways associated with the development and progression of CRCHM, which might be altered in metastatic lesions.

To date, in the liver three subtypes of adrenoceptors belonging to the super family of G-protein coupled receptors (GPCR) have been identified: β2-, α1- and α2-ARs [20–22]. Each subtype couples to a different signal transduction system. β2-ARs couple to stimulatory G-protein (Gs) and upon receptor stimulation activate AC to generate cyclic AMP (cAMP) from ATP [23]. α2-receptors couple to the inhibitory G-protein (Gi) thus inhibit intracellular cAMP-mediated signalling processes [24]; α1-ARs couple to phospholipase C over Gq/11-protein and mediate phosphoinositide turnover and calcium mobilization [25]. Among various subtypes of adrenergic receptors, which have been identified pharmacologically, as well as by molecular cloning, two subtypes play a major role in the control of hepatic metabolic function in humans [26]. The cAMP-linked β2-AR, as the predominant receptor subtypes in the human liver, mediate the major metabolic events, whereas the role of calcium-linked hepatic α1-ARs is still a matter of debate [13]. In addition, it is still not yet known to what extent metastatic lesions in the liver are associated with hepatic dysfunction of the autonomic adrenergic control; or whether this malignant transformation in the liver enhances or impairs the function of the β2-AR-G-protein-AC-system.

Thus, since in the human liver the β2-AR is the sole receptor-subtype through which catecholamines mediate their metabolic effects [12,27], identifying and characterizing the specific β-AR subtype in the liver with CRCHM may significantly enhance our understanding about the metabolic consequences of metastatic invasion. In the present study, we investigated the β-AR-G-protein-AC-system in livers with CRCHM, and contrasted them with contra-lateral, non-adjacent and non-metastatic liver specimens. Moreover, we examined β-AR expression in primary colon cancer and in normal colon, and compared the magnitude of β-AR alteration in primary colon cancer and secondary metastatic tumors.

2. Material and methods

2.1. Patients

The experimental procedures and the purposes were thoroughly explained to all subjects, and written consent was obtained. The study protocol was approved by the Ethical Committee of the University of Leipzig in accordance with the federal law of Germany. Liver specimens were obtained from 13 patients (7 men and 6 women, median age 63 years, range 46–76 years old) who underwent curative liver resection for colorectal cancer hepatic metastasis. Pre-operative and intra-operative assessment demonstrated that all patients had three or fewer metastases localized on one side of the liver, and no extra-hepatic diseases were documented. Histopathological records were available for all patients, and hence, the diagnosis of CRCHM could be confirmed in all patients. Patients with chronic liver disease including viral hepatitis or previous history of viral hepatitis and liver cirrhosis were excluded from this study. Samples taken from patients with histologically confirmed steatosis (if greater than 10% of hepatocytes) were also excluded. All participants underwent an examination of health status established by medical history to exclude or include previous medication and other diseases. Primary disease was well controlled, and the median length of time between surgery for colorectal cancer and hepatectomy was 12 months (range 1–46). None of the patients had received catecholamines or other drugs known to interact with adrenergic receptors. 3 to 5 g metastatic liver tissue samples were taken from resected specimens, and 1 g non-metastatic liver tissue was obtained from the contralateral non-adjacent, non-metastatic lobe. For comparison, samples of carcinoma from primary colon cancer and non-adjacent non-tumor tissue were taken from five other patients. After carefully discarding necrotic parts of metastatic and cancerous tissues, tissue samples were immediately frozen in liquid nitrogen until use.

2.2. Preparation of liver membranes

For the assessment of the surface β-AR density, receptor subtypes as well as the receptor-G-protein coupling, liver plasma membranes were prepared according to the method of Wolfe et al. [28] with slight modifications. All subsequent procedures were performed at 4 °C. Frozen liver samples were thawed and finely minced with scissors in 20 volumes of ice-cold lysis buffer containing 20 mmol/l NaHCO3, and the slurry was homogenized using motor-driven Teflon pestle homogenizer (Polytron, Brinkmann Instruments,) with 10 strokes at 1500 rpm. To sediment connective tissues and cell debris, the homogenate was centrifuged at 500×g for 10 min. The crude homogenate was then strained through four layers of cheesecloth. The supernatant was centrifuged at 40,000×g (Beckman Ultra-Centrifuge) for 30 min. The pellets were washed once again by re-suspending (Polytron) and gentle homogenization in a lysis buffer followed by re-sedimentation at the same speed as before. The final pellets were resuspended and dispersed (homogenizer) in an incubation buffer (containing 10 mmol/l Tris buffer, pH 7.4, 154 mmol/l NaCl, 0.55 mmol/l ascorbic acid) to obtain a final protein concentration of 0.5–1 mg/ml, aliquoted, and snap frozen in liquid nitrogen, and stored at −70 °C. Colon membranes were also prepared as described above.

For the determination of adenylate cyclase (AC) activity, crude membranes were prepared from NA-NM and CRCHM livers after homogenization with a motor-driven Teflon pestle homogenizer as described above, with the difference being that the final pellets were resuspended in TEN buffer (consisting of 20 mmol/l Tris-base, pH 7.4, 25 °C, 25 mmol/l NaCl, 1 mmol/l EDTTA). Before determining the AC activity, membrane pellets were obtained after centrifugation at 2000×g for 10 min.

The protein concentration was determined with Folin reagent according to the method employed by Lowry et al. [29] using bovine serum albumin as the standard.

2.3. β-adrenoceptor binding assays and sub-typing

The β-AR density was determined in plasma membranes by the methods recently described by Abraham et al. [30]. The overall strategy was to assess the binding capabilities of the β-adrenoceptor antagonist (∼)-[125I]-iodocya-
nopindolol (ICYP) to membrane preparations from tumor-free and non-adjuvant tumor-bearing tissue samples of each patient, thereby, alterations in maximal receptor density ($B_{\text{max}}$) or receptor affinity to the ligand ($K_D$) could be detected. In brief, membrane suspensions (20 µg) were incubated in duplicates with increasing concentrations of ICYP (ranging between 5 and 200 pmol/l) in a total volume of 250 µl. The incubation was carried out for 1.5 h at 37 °C. Non-specific binding was defined as bound radioligand (ICYP) in the presence of 1 µmol/l (±)-CGP-12177 (non-labelled β-AR antagonist). The reaction was terminated by the addition of 10 ml ice-cold buffer containing 10 mmol/l Tris buffer, pH 7.4, and 154 mmol/l NaCl. Free radioactivity was separated from bound radioligand by a rapid filtration over GF-52 glass fiber filters (Schleicher and Schuell, Germany) using a 1225-sampling manifold (Millipore Corp., Schwalbach, Germany). Each filter was washed once with an additional 10 ml of washing buffer. The radioactivity of ICYP was counted with a gamma counter (1470 WIZARD Automatic Gamma Counter, Perkin-Elmer Life Sciences). Specific ICYP binding was defined as the difference between total and non-specific ICYP binding.

In sub-typing β-ARs in the human liver, competition binding studies were performed in membrane preparations (20 µg) with constant ICYP concentration (~80 pmol/l), but varying concentrations of highly subtype-selective β-AR antagonists. Here, the highly β2-selective antagonist ICI 118,551 and the highly β2-selective antagonist CGP 207 12A with increasing concentrations (10$^{-3}$–10$^{-4}$ mol/l) were used. All other subsequent procedures (incubation, stopping the reaction, separation of the radioactivity, defining non-specific binding) were carried out as described above.

2.4. Agonist competition binding assays

β-adrenergic agonist binding was evaluated in membranes of both NA-NM and CRCHM liver samples, in order to proof the existence of multiple receptor affinity states resulting from conformational changes through G-protein interaction. Aliquots of membrane suspension (20 µg) were mixed with 50 µl of the incubation buffer (see above) and 50 µl of ICYP (~80 pmol/l) to determine total receptor binding. To assess the number of receptors in the high and low affinity state, ICYP was displaced with increasing concentrations of the β-AR agonist isopropenol (0.1 nm–100 µM final concentrations) in the absence or presence of 100 µmol/l guanosine 5′-triphosphate (GTP). Non-specific binding was determined in the presence of 1 µM (±)-CGP-12177. Equilibration, harvesting and counting were carried out as described in the previous section.

2.5. Adenylate cyclase assay

Assays were carried out according to the methods of Salomon et al. [31] with slight modifications as described elsewhere [30]. In brief, aliquots of membranes (30–40 µg protein) were incubated for 10 min at 37 °C. The reaction mixture contained final concentrations of 40 mmol/l HEPES, pH 7.4, 5 mmol/l MgCl$_2$ and 1 mmol/l EDTA 0.5 mmol/l [α-32P]-ATP (about 1000 000 cpm/tube), 0.1 mmol/l cAMP, 0.5 mmol/l ATP and a creatine phosphokinase-ATP-regenerating system (containing 5 mmol/l phosphocreatine and 0.5 mmol/l creatine phosphate, creatine phosphokinase and cyclic adenosine 3′, 5′-triphosphate (cAMP), forskolin, NaF and bovine serum albumin from Sigma-Aldrich). ATP, adenosine deaminase, creatine phosphate, creatine phosphokinase and cyclic adenosine 3′, 5′-monophosphate (cAMP) were from Roche Diagnostics (Mannheim, Germany). All other chemicals were of analytical grade and commercially available.

3. Results

3.1. Malignancy induced alteration in β-adrenoceptors

Membranes were prepared from non-metastatic, non-adjacent and metastatic liver tissue of the same patient, and the β-AR density ($B_{\text{max}}$) and the receptor affinity for the ligand ($K_D$) were measured from saturation binding studies using the labelled β-adrenoceptor antagonist (–)-[125I]-iodocyanopindolol (ICYP). The saturation ICYP binding data are depicted in Fig. 1, and for both parameters mean values are summarized in Table 1. In both NA-NM and CRCHM livers, the specific ICYP binding was saturable and of high affinity with a dissociation constant ($K_D$) of 12.29 ± 1.26 and 12.57 ± 1.33 pmol/l, respectively (Table 1). Thus, the affinity of ICYP binding to the plasma membrane remained unaffected during metastatic invasion. The characteristic ICYP binding pattern suggests, furthermore, in both tissue types, the presence of an apparently homogeneous population of high affinity binding sites. This could be confirmed by the fact that the Scatchard analysis for specific ICYP binding yielded a linear line; a single component of binding sites in both plasma membranes (Fig. 1, inset). The density of β-ARs ($B_{\text{max}}$) in CRCHM liver membranes was significantly decreased compared to NA-NM-liver tissues (40.09 ± 2.83 vs. 23.09 ± 3.24 fmol/mg protein; $P < 0.001$).

For comparison, whether the β-AR expression might be altered in the primary tumor, we assessed ICYP binding sites in...
membranes obtained from nonadjacent/non-tumor colon tissues and in colon with the primary tumor. Here, we demonstrated a concomitant significant decrease in the density of β-ARs in CRC tissues (normal colon vs. CRC: 37.60 ± 2.20 vs. 23.80 ± 3.50; \( P < 0.01 \)), without alteration in the receptor affinity for ICYP \( (23.10 ± 2.8 \text{ vs. } 17.10 ± 3.20) \) (Table 1). However, in general, the total number of β-ARs in colon tissue was slightly lower than in the liver. The decrease in \( B_{\text{max}} \) in colorectal cancer hepatic metastasis (CRCHM) was paralleled with the decrease in the colon with cancer, thus, suggesting that the tumor-bearing cells were similarly altered, and that during malignant transformation, β-ARs in both tissues might have been internalized from surface membranes to the intracellular sites to a similar extent.

In a further series of experiments, in identifying and characterizing β-AR subtypes in membranes of NA-NM liver tissues and in liver with CRCHM, we have discriminated the ICYP binding with increasing concentrations of ICI 118,551, a highly \( \beta_2 \)-selective antagonist, and of CGP 20712A, a highly \( \beta_1 \) selective antagonist. The ICYP competition binding curves for ICI 118,551 were in both tissue types monophasic, of high affinity, and had a Hill coefficient of unity (Fig. 2) best fitted to one-site binding model. The \( K_i \) values for ICI 118,551 obtained from membranes of NA-NM liver were within the same range as in the liver with CRCHM (Fig. 2, inset), indicating the high affinity ICI 118,551 binding sites in the human liver are principally of only one population of β-ARs, i.e. the \( \beta_2 \)-subtype. Replacement of ICI 118,551 by the \( \beta_1 \)-selective antagonist (CGP 20712A) gave concentration-dependent ICYP-inhibition curves with lower affinity than for ICI 118,551, but same proportion (≈100%) of the \( \beta_2 \)-subtype could be obtained (data not shown). The highly selective \( \beta_2 \)-adrenoceptor antagonist ICI 118,551 [35] was about 1000 times more potent in inhibiting ICYP binding than the highly selective \( \beta_1 \)-adrenoceptor antagonist CGP 20712A [36]. In CRCHM, neither the binding pattern nor the inhibition constant \( (K_i) \) of either antagonists were affected, suggesting that CRCHM did not cause a shift to other subtypes of the β-adrenergic receptors.

### 3.2. Changes in \( \beta \)-adrenoceptor-G-protein coupling efficiency

It has been shown in several G-protein coupled receptor systems that the agonist competition curves (with antagonist radioligands) shift to the right, to low affinity binding states, and become steep when tissues are pre-incubated with guanine nucleotides, whereas antagonist displacement curves are not affected [37,38]. Thus, we assessed the effects of guanosine 5′-triphosphate (GTP) on the inhibition of ICYP binding by isoproterenol in NA-NM and CRCHM liver tissue membranes, and evaluated the number of β-ARs in the high affinity state, which couple to stimulatory G-protein \( (G_S) \) in response to β-AR stimulation. With the high affinity state, receptors couples to stimulatory G-protein regulatory processes, whereas receptors with a low affinity state are apparently uncoupled from \( G_S \).
Isoproterenol displaced ICYP binding in a concentration-dependent manner with two binding sites of high and low affinity state of the \( \beta \)-ARs, and the curves were significantly better fitted for the two-site than the one-site binding model (F-ratio: \( P < 0.05 \). Figures not shown) with a Hill coefficient less than unity (1.0). As illustrated in Table 2, \( \sim 53\% \) of high affinity ICYP binding sites in NA-NM liver and lower than 10\% in CRCHM liver could be calculated from isoproterenol inhibition curves. In CRCHM liver, isoproterenol competition curves were monophasic, with predominantly low affinity binding sites (above 90\%). In NA-NM liver, the \( K_{iH} \) value for isoproterenol at high affinity state was 18.29 \( \pm \) 3.91 nmol/l, whereas in CRCHM liver, the \( K_{iH} \) value amounted to 871 \( \pm \) 54 nmol/l. The \( K_{iL} \) value at low affinity state was 707 and 1084 nmol/l in NA-NM and CRCHM, respectively. Taken together, the high affinity state of isoproterenol in CRCHM resembled that of NA-NM liver at low affinity state; thus, \( \beta \)-ARs in CRCHM were largely uncoupled from the stimulatory G-protein.

On the other hand, in NA-NM liver, in the presence of 100 \( \mu \)M GTP, the isoproterenol competition curves were shifted to the right, into steepened monophasic curves with low affinity (\( K_{iL} \): 913 \( \pm \) 132 nmol/l; Table 2). This low affinity was within a similar range when compared to \( pK_{iL} \)-value of isoproterenol without GTP (\( pK_{iL} \): 707 \( \pm \) 44 nmol/l). In contrast, in CRCHM liver, there was obviously no modulation of the concentration-dependent isoproterenol-inhibition curves by GTP, since the majority of \( \beta \)-ARs was found to be at low affinity state. There was no significant difference between \( K_{iH} \) values of isoproterenol in the presence or absence of GTP in CRCHM liver. The complex of \( \beta \)-ARs and GDP-bound Gs-protein exhibits high affinity for agonists, whereas the receptor alone shows low affinity [39,40]. Thus, this suggests that the excess of GTP causes the exchange from GDP to GTP on Gs-protein, the release of Gs from the receptors, and the resultant low affinity of free receptors for an agonist.

### 3.3. Changes in adenylate cyclase activity

In the end, we performed a downstream characterization of the hepatic \( \beta \)-AR coupling to the enzyme AC, which converts ATP to the second messenger cAMP, in membranes of NA-NM and CRCHM liver. As shown in Fig. 3 and summarized in Table 3, the basal AC activity was identical in all tissue membrane preparations (in NA-NM livers 3.46 \( \pm \) 0.37 and CRCHM livers 3.88 \( \pm \) 0.71 pmol cAMP/mg protein/min). Concomitant to the significant down-regulation of the hepatic \( \beta \)-ARs in CRCHM livers, CRCHM elicited a significant desensitization of \( \beta \)-AR-mediated AC activity, as measured by the responsiveness to isoproterenol in the presence of GTP. The decreased AC stimulatory effect of GTP suggests impaired coupling efficiency of the receptor to G-proteins. Moreover, substances, which directly activate the catalytic units of the enzyme, induced substantial decrease in AC activity. Thus, the addition of forskolin significantly reduced the net activity of the catalytic unit of AC in membranes of CRCHM livers (537\% AC above basal in control livers vs. 205\% AC above basal in CRCHM livers (\( P < 0.001 \), Table 3). Similarly, NaF-mediated stimulation of AC was significantly reduced in CRCHM livers when compared to NA-NM livers (\( P < 0.001 \), Table 3). NaF causes maximal activation of all G-proteins. The similar range of decrease in AC activation by all stimulants confirmed that the increases in G-protein-mediated total AC

![Fig. 3. Adenylate cyclase activity in NA-NM and CRCHM liver membranes.](image)

Ordinates: net increase in hepatic adenylate cyclase activity upon stimulation in pmol cAMP formed/mg protein/min (for details, see Materials and methods).

The response was measured under basal conditions and in the presence of 10 \( \mu \)M GTP plus 10 \( \mu \)M isoproterenol, 10 \( \mu \)M forskolin or 10 mmol/l NaF. Data represent mean \( \pm \) S.E.M. (\( n = 13 \)) determined in triplicates. ***\( P < 0.001 \) vs. NA-NM liver. Analyzed data and percent decrease in AC activity are summarized in Table 3.
catalytic activity were able to offset desensitization of the β-AR signal-transduction pathways in CRCHM livers.

4. Discussion

In the present study, the biochemical properties and function of β-adrenoceptors in CRCHM and NA-NM livers were investigated with respect to receptor concentration, pattern of distribution, affinity of the receptor to G-protein coupling as well as AC response to different activating agents. Previously, it has only been shown that adrenergic receptors might be affected in patients with hepatocellular carcinoma: increased β2-ARs as well as AC activity have been described [41]. Main findings of our study were that in CRCHM: (1) the density of β2-ARs was altered, but not the affinity of the receptors to the corresponding ligand; (2) in both CRCHM and NA-NM livers, only the β2-AR subtype which functionally couples to Gs-protein and AC was expressed, and CRCHM did not influence the subtype distribution; (3) the total number of β2-ARs with high affinity – which are known to couple to the signal-transducting G-protein – were decreased; (4) AC activity was reduced in liver membranes with CRCHM. Furthermore, we compared in the β-AR expression in the liver with that in colon bearing the primary tumor (colon cancer) versus normal colon—whether there exists a similarity or dissimilarity with the metastatic tumor in the liver. The β-AR density was markedly decreased as similar as in CRCHM in primary colon cancer.

The apparent dissociation constant (K_D) for ICYP β-AR binding was unaltered in all cases. This indicates that the affinity of the receptor at least for this ligand was not affected in the colon and liver during malignant transformation. Thus, metastatic tumor cells in the liver from colorectal cancer and primary colorectal carcinoma do seem to similarly, and largely affect the β-AR binding sites in both tissues. Our results were in agreement with the altered hepatic adrenergic receptors in the rat lymphosarcoma model [19]. However, there are also other conditions, which differentially regulate adrenergic receptor expression. For example, increased β-AR density has been reported in hypothyroid [42], adrenalectomy [43] as well as in rat liver after cholestasis [44]. Therefore, it seems likely that other receptor regulatory mechanisms such as hormones might have been involved in such conditions.

The underlying mechanisms responsible for β2-AR down-regulation from surface membranes in the human colon and liver affected by the same type of cancer are not yet clear. However, two possible explanations for these results may be considered. First, it was suggested that the hepatic sympathetic nervous activity is enhanced in partially hepatectomized rats [45], in patients with severe haemorrhage [46] and in patients with decompensated liver cirrhosis [47,48]. These conditions might cause increased levels of catecholamines, thus, might impair the β-AR system in the liver. Although the functional implication of adrenergic receptor-mediated neuronal mechanisms is likely to be associated with catecholamine release, it is yet unclear whether there is chronic increase in circulating catecholamines in patients with CRCHM. However, it is well known that prolonged stimulation of the receptor by catecholamines or any synthetic analogues such as β-adrenoceptor agonists frequently results in a time-dependent decrease in functional responsiveness (desensitization, tachyphylaxis, refractoriness) of the receptor-G-protein-AC-complex [49–51]. A second hypothesis, which might help to cast some light on the explanation of the reduced β-ARs in CRC and CRCHM, would be the malignancy related elevation of carcinoembryonic antigen (CEA) and induction of pro-inflammatory cytokines such as IL-1α, IL-6, IL-10, and TNF-α in Kupffer cells [52–56]. These factors are supposed to facilitate colorectal cancer hepatic metastasis. If both catecholamines and pro-inflammatory cytokines are largely released during the metastatic process, they might play an important role in initiating the progress of malignancy in the liver, due in part, to impairment of the β-AR signal transduction system by chronic receptor stimulation.

Furthermore, we have demonstrated in the human liver the presence of only the β2-AR subtypes. From concentration-displacement experiments using the β2-selective antagonist ICI 118.551, we obtained a ratio of 98:2% (β2:β1) [35]. Moreover, in both NA-NM and CRCHM liver tissues, the concentration–inhibition curves of ICI 118.551 remained unchanged; indicating that in the liver with CRCHM, the β2-subtypes are consistently expressed and hepatic receptor subtype distribution is unaltered during carcinogenesis, thus, the decrease in the receptor number can be attributed only to the β2-subtype.

Another major finding of the present study was the markedly decreased number of the β2-ARs in the ‘high affinity state’ (i.e., conformation of the receptor that is essential for coupling stimulated receptor to the Gs-protein-AC system [50]), suggesting diminished functional responses of β2-ARs upon Gs stimulation in CRCHM. As a classical picture of G-protein coupled receptors (GPCR), (−)-isoproterenol inhibited ICYP binding with concentration–inhibition curves that better fitted to a two-site than one-site binding model. The biphasic isoproterenol concentration curves, as these were the case in NA-NM liver, thus comprised β-ARs in the ‘high-affinity state’ (40–52%) and the remaining ‘low-affinity state’, the latter which correlates to receptor states uncoupled to the heterotrimeric G-protein. However, in CRCHM the amount of β-ARs in the ‘high-affinity state’ was reduced by 90%. Moreover, our data demonstrated that the addition of GTP triggered a great reduction of the high-affinity (−)-isoproterenol binding sites with the concomitant increase in low-affinity state in NA-NM liver, while additional GTP had almost no effect on (−)-isoproterenol competition curves in CRCHM liver. In sum, as a first report, it means that large number of β2-ARs detected in CRCHM liver have lost functional coupling to Gs-protein, and that a defect in the signalling cascade is an intrinsic component of metastatic transformation. Our data are, at least in part, consistent with findings demonstrated in other pathological conditions, e.g., in heart failure, that the number of high affinity binding sites decrease in a similar but to a lesser extent [57].

To better understand the β-AR signal transduction system, we have correlated the functional coupling efficiency of the
receptors to AC cascade in membranes from NA-NM and CRCHM livers. Accordingly, the AC activity was assessed: (a) by stimulating the β-ARs (using the β-AR agonist isoproterenol), (b) by non-receptor-mediated activation of the enzyme with GTP (acting on G\textsubscript{S} and G\textsubscript{\alpha-protein}), with forskolin (activates predominantly the catalytic unit of the AC, but partly also G\textsubscript{S} \cite{58} and with NaF (stimulates G\textsubscript{S}) \cite{59}. The basal AC activity was not different between both NA-NM and CRCHM liver membranes. In CRCHM, however, the activity of AC decreased markedly in response to all activators. These results document that in CRCHM, AC was not only impaired by agents acting on β-ARs (isoproterenol), reflecting that AC is functionally uncoupled and is not recruitable through β-AR stimulation \cite{60}, but also to agents which bypass the receptor and act on post-receptor levels (GTP, forskolin, NaF). This indicates that the AC response cannot be enhanced by stimulating G\textsubscript{S}-protein coupling and directly by augmenting the activity of the catalytic unit of AC in CRCHM. The decrease in G\textsubscript{S}-mediated (GTP, NaF) synthesis of cAMP was an indirect indices of the ‘uncoupled’ β-AR to G\textsubscript{S}-protein (discussed above).

In summary, in patients with CRCHM the whole set of hepatic β-AR-G-protein(s)-adenylate cyclase system was blunted. The mechanisms underlying this altered β-AR signalling responses remain unclear. It might be due to uncoupling of the receptor or to an inhibition of the receptor by malignancy or cancer related mediators. A better understanding of malignancy related alterations in this system might direct new strategies for new patient management and therapeutic options to minimize cancer related complications.

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