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Midgut carcinoids; surgical aspects, biogenic amines and vascular effects

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Document Version

Publisher's PDF, also known as Version of record

Publication date:
2006

[Link to publication in University of Groningen/UMCG research database](#)

Citation for published version (APA):

Vries, H. D. (2006). *Midgut carcinoids; surgical aspects, biogenic amines and vascular effects*. Eburon.

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Chapter 6a

DIMINISHED BAROREFLEX SENSITIVITY IN CARCINOID PATIENTS WITHOUT SIGNS OF EARLY ATHEROSCLEROSIS OR ENDOTHELIAL DYSFUNCTION.

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Submitted

Abstract

Serotonin or other vasoactive amines produced by a carcinoid tumor are thought to be responsible for the characteristic changes in the mesenteric vessels known as vascular elastosis. The aim of this study was to evaluate the structural and dynamic properties of the vessel wall of carcinoid patients outside the mesentery as compared to healthy controls.

In 17 carcinoid patients with elevated platelet serotonin level and 21 healthy age and sex matched volunteers the intima-media complex thickness (IMT) of the common carotid artery as a marker of early atherosclerosis, and flow-mediated dilation (FMD) of the brachial artery to assess endothelial function were determined. Baroreflex sensitivity (BRS) as function of autonomic modulation and regulation of vessel tone was measured using transfer function analysis of the Finapres® signal.

No differences were found in IMT or FMD between the two groups, suggesting no structural or functional alterations in the brachial and carotid artery in carcinoid patients. The BRS, however, was lower in the carcinoid group (1.5 ± 0.3 msec/mmHg) versus controls (2.1 ± 0.5 msec/mmHg, $p < 0.0001$) indicating an overbearing sympathetic system. In conclusion, the degree of BRS reduction may indicate an increased risk for cardiac events.

Introduction

A carcinoid tumor usually is a relatively slowly growing tumor originating from enterochromaffin cells. Especially the midgut carcinoids are able to produce various biogenic amines, among which serotonin and its metabolite 5-hydroxyindoleacetic acid (5-HIAA). Serotonin is thought to be responsible for the characteristic vascular changes in the mesentery known as vascular elastosis, which is pathognomonic for carcinoid disease. It consists of fibrosis of media and adventitia of mesenteric vessels causing narrowing of the lumen.^{1,2-4} Abdominal angina is a known complication in advanced stage carcinoid disease.⁵ The abdominal pain can be reduced with sublingual medication e.g. nitroglycerin, indicating a dynamic component in the mesenteric ischemia which might be due to endothelial dysfunction.⁶ Elevated serotonin levels will eventually lead to carcinoid heart disease. This disorder is usually located within the right heart, consisting of fibrous depositions in the endocardium and the valves leading to valve insufficiency, cardiac failure and cardiac death.⁷ Measurement of the intima media complex thickness (IMT) of carotid or femoral vessels has in recent years become a generally accepted method to assess structural abnormalities in larger arteries and to detect early atherosclerosis. Besides structural changes in the vessel wall several reports indicate functional changes due to circulating biogenic amines, presumably serotonin.⁸⁻¹⁰ Since the original article of Celermajer and Deanfield in 1993, flow-mediated dilation of the brachial artery has become an important method to assess endothelial function.^{11,12} Several articles have shown impaired flow-mediated dilation in smokers, patients with hypertension or diabetes or some of the other conditions associated with increased cardiovascular risk.¹³ Vascular function includes not only endothelial function, but also autonomic modulation and regulation of vessel tone. These functional properties reflecting cardiac autonomic nervous balance can be assessed by measuring the baroreflex sensitivity (BRS). The primary purpose of the arterial baroreflex is to keep blood pressure close to a particular set point over a relatively short period of time via a negative feedback system, counteracting transient changes in blood pressure. A sudden fall in blood pressure will trigger an autonomic response in the baroreceptors situated mainly in the aortic arch and carotid artery in order to increase heart rate and cardiac contractility with the purpose to regain blood pressure. An impaired BRS is an independent risk factor for sudden death after myocardial infarction.^{14,15} In hypertensive humans and animals, the baroreflex control of heart rate is diminished.¹⁶ Besides the pathognomonic morphological changes in mesenteric vessel wall, there are no data on endothelial dynamic and

autonomic function or on structural disorders of the peripheral vessel wall in carcinoid disease even when such functional and structural vascular changes may have prognostic cardiovascular relevance. In this study these aspects were studied in carcinoid patients and healthy volunteers.

Patients and methods

From September 1999 to January 2000, all consecutive midgut carcinoid patients with platelet serotonin levels of 3 times the upper reference limit and higher during prior visits, visiting our outpatient clinic were invited to participate in this study. Patient's history was obtained regarding diabetes, cardiovascular disease and hypertension. From August 2003 to February 2004, controls matched for sex and within the age range with the patient group were recruited. In none of the controls manifest cardiovascular or renal disease, hypertension or diabetes was present. The study was approved by the Medical Ethical Committee. All patients and volunteers gave informed consent.

Carotid Intima-Media Thickness (IMT) measurements

IMT measurements were performed using a Pie Medical Scanner 200 device with a linear array transducer of 7.5 MHz. The IMT was measured at the posterior wall of the left common carotid artery approximately 1 cm proximal to the bulbous at 3 different positions. A B-mode image was obtained of the carotid artery after which a M-line was positioned perpendicular to the posterior wall, showing an intima-media complex. The radio frequency signals and the electrocardiogram were stored on hard disk for 3 periods of 4 sec and IMT was calculated for the total 4-sec periods. The recorded files were processed using the wall thickness calculation section of the Wall Track System 2.0 software (Pie Medical, Maastricht, The Netherlands). The mean of the 3 measurements was used to calculate IMT. Technicians blinded for patient characteristics performed all off-line analyses. The intra-observer variability in our laboratory is 0.051 mm, or 8.1% of the mean IMT, and is independent of wall thickness ($R^2=0.13$, non-significant)

Flow-mediated dilation (FMD) procedure

The method is based on ultrasonography of the brachial artery to assess endothelium-dependent and -independent function. The measurement system consisted of an ultrasound scanner (Scanner 200, Pie Medical), and a personal computer with a high-speed data acquisition board, frequency sample 21.5 MHz. Dedicated software (Wall Track System 2.0, Pie Medical) was used to measure and analyze changes in brachial artery

vessel diameter. Using a 7.5 MHz transducer the brachial artery was visualized. A two-dimensional longitudinal B-mode image of the brachial artery was obtained. The radiofrequency (RF) signals from the M-mode output were relayed to the wall tracking system and stored digitally. Using the RF signal the anterior and posterior vessel wall are identified and marked. Vessel wall movements are tracked using off-line analysis. This enabled measurement of end-diastolic diameter for each beat. Measurements were conducted in supine position at a constant room temperature. A custom built holder was used to stabilize the probe during the measurements. Procedures were as follows: (1) two brachial artery baseline diameter measurements (2) arterial occlusion by inflation of a pneumatic tourniquet placed around the forearm distal to the segment of artery scanned (3) deflation of the tourniquet after 4 min, resulting in an increased blood flow to the distal part of the forearm inducing an endothelium-dependent vasodilatation (4) measurement of the brachial artery diameter after deflation continuously for 6 cycles of 22 sec (5) measurement of the brachial artery 2.5 and 5 min after giving nitroglycerin 0.4 mg sublingually, resulting in endothelium-independent vasodilatation. For each measurement consisting of 22 sec data acquisition the average end-diastolic diameter of these 22 sec was used. Intra- and inter-observer variability of this system is 2.5% and 5.0%, respectively. The off-line data analysts were blinded for the clinical parameters of the subjects. FMD was calculated as the percent maximal increase in arterial diameter during hyperemia compared to the average of 2 baseline diameters, and nitroglycerin induced dilation was calculated the same way for the maximal post-nitroglycerin diameter.

BRS measurements

BRS was investigated using Finapres® equipment (Ohmeda TM2300, Inglewood, Co, USA). Patients were asked not to drink coffee or smoke prior to the investigations. A Finapres cuff was applied to the midphalanx of the third finger for continuous beat-to-beat blood pressure measurement.

In short, the BRS was determined by the transfer function technique using the CARSPAN program (IEC ProGamma, Groningen, the Netherlands), as described previously.^{17,18} This program allows discrete Fourier transformation of non-equidistant samples of blood pressure and RR interval series. After correction for artifacts and checks for stationarity, BRS is defined as the mean modulus between spectral values of systolic blood pressure variability and heart rate variability in the low frequency

(LF, 0.07-0.15 Hz) power spectrum band with at least 0.3 coherence, expressed in ms/mmHg.

Statistics

Statistical analysis of the data of the two groups was performed using the independent sample t-test. BRS values are given as natural logarithms. Only p-values <0.05 were considered significant.

Results

Eighteen consecutive midgut carcinoid patients meeting the inclusion criteria were invited to participate. One patient refused, 17 gave informed consent, 10 males and 7 females. Median age was 60 (range 51-73) years. The median platelet serotonin at time of the investigation was 24 nmol/10⁹ platelets (range 17-39) and the median urinary 5-HIAA excretion 23 mmol/mmol creatinine (range 8-324). The median reported time of symptoms of disease prior to the investigation was 10 years (range 3-18). None of the patients had a history of diabetes, cardiovascular disease or hypertension prior to the onset of the carcinoid disease. The control group consisted of 21 healthy volunteers, median age 58 (range 43-73) years and were sex matched.

Common carotid Intima Media Complex

The mean IMT of the far wall of the left carotid artery in carcinoid patients was similar to that of control namely 0.70 mm ± 0.11 and 0.75 mm ± 0.17 respectively (p=ns).

Flow mediated and nitroglycerin-induced dilation of brachial artery

For this parameter there were also no differences between the carcinoid patients and the controls (Table 1). The mean diameter of the brachial artery was 4.9 mm ± 1.2 in carcinoid patients compared to controls 5.1 mm ± 0.8 (p=ns). After occlusion the mean diameter was 5.1 mm ± 1.1 and 5.3 mm ± 0.7 respectively. The mean percentage dilation after occlusion was 4.7% ± 7.5 and 6.9% ± 8.7 respectively (p=ns). Compared to the non-endothelial dependent dilation (i.e. after sublingual nitroglycerin), there was no difference between the groups either. In carcinoid patients the dilation after occlusion was 95.3% ± 5.9 of the dilation after nitroglycerin administration, in controls 92.2% ± 6.7 (p=ns).

Baroreflex Sensitivity

The mean BRS in carcinoid patients was 1.5 ± 0.3 msec/mmHg and in the controls 2.1 ± 0.5 msec/mmHg ($p < 0.0001$, Table 1). In the carcinoid group no correlation was found between serotonin in platelets or urinary 5-HIAA excretion and the BRS. There were no differences in blood pressure between the two groups

Parameter	Carcinoid N=17	Controls N=21	p-value
CCA intima-media thickness (mm)	0.699 ± 0.113	0.751 ± 0.168	0.56
Baseline BA diameter (μm)	4920 ± 1234	5052 ± 797	0.78
Flow-mediated vasodilation (%)	4.7 ± 7.5	6.9 ± 8.7	0.78
NTG-mediated vasodilation (%)	4.7 ± 5.9	7.8 ± 6.7	0.09
Baroreflex sensitivity (msec/mmHg)	1.51 ± 0.34	2.11 ± 0.48	<0.001

Table 1: Vascular structure and functional parameters in carcinoid patients and controls. CCA: common carotid artery; BA: brachial artery; NTG: nitroglycerin; all values are means \pm SD.

Discussion

To our knowledge this is the first study on both structural and functional characteristics of peripheral arteries in carcinoid patients. The BRS in the carcinoid patients is markedly diminished compared to sex and age matched controls.

However, no differences in carotid IMT or FMD were found. There is abundant literature pointing out a low value BRS as an unfavorable prognostic factor in post-myocardial infarction patients and as independent risk factor of cardiac failure.^{14-16,19,20} For other patients like those with chronic kidney disease a low BRS also acts as a risk factor for cardiac events.²¹ Our results suggest that carcinoid patients suffering from high levels of serotonin are at risk of arrhythmia and sudden cardiac death. We hypothesized that the well known vascular elastosis in mesenteric arterial and venous vessels in carcinoid patients could also be associated with both structural and functional abnormalities in the systemic vasculature. Remarkably however, no differences were found in either carotid IMT, or in FMD of the brachial artery as a marker of endothelial function between carcinoid patients and controls. Vascular elastosis is predominantly present in mesenteric arteries and veins.^{1,2,4} Although it is generally considered to be the result of serotonin released from the tumor there is no explanation why just mesenteric arteries (which are

efferent to the tumor) and not extra-mesenteric arteries are involved. Several authors suggested local factors inducing local vascular fibrosis.^{22,23} In 2004 Modlin et al surveyed the literature over the last 40 years covering the incidence, diagnosis, therapy and biological basis for carcinoid-associated fibrosis. They conclude that the mechanism of fibrosis is still poorly understood and there are no means by which this complication can be predicted or monitored.²⁴ The present study in carcinoid patients with high circulating serotonin levels tends to support the theory that vascular elastosis in patients with a midgut carcinoid is a local, rather than a systemic problem.

Increasingly data emerge that heart failure due to carcinoid heart disease is not merely a result of fibrotic plaques in the right side of the heart but also the left side, pericardial effusions and cardiac metastases.²⁵ Presumably loss of vasomotor control in carcinoid disease is caused by alterations in pre- and postsynaptic receptor configuration resulting in an impaired cardiac autonomic nervous function. Future investigation elucidating the cause of the BRS impairment in carcinoid patients should focus on autonomic dysregulation, rather than on structural morphologic changes in the vessel wall.^{26,27}

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Chapter 6b

LOSS OF PRE-SYNAPTIC SEROTONIN VASOCONSTRICTOR CONTROL IN ISOLATED POPLITEAL ARTERY PREPARATIONS FROM A PATIENT WITH MIDGUT CARCINOID

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Abstract

Metastatic carcinoid disease can be associated with vasomotor instability causing flushes and hypotension or hypertension. In addition morphological changes in the vessel wall (i.e. vascular elastosis) with thickening of the intima-media complex resulting in reduced compliance can occur. Pharmacological studies on isolated arteries from carcinoid patients have not yet been performed. Therefore this study investigates functional alterations in serotonin response in isolated popliteal artery preparations of a carcinoid patient. The effect of pre-synaptic inhibition on serotonin induced contraction and the involvement of serotonin-2A (5-HT_{2A}) receptors in these responses as well as the role of contractile prostaglandins was analyzed.

Methods: In a metastatic carcinoid patient a below knee amputation was performed because of necrosis due to ischemia. The popliteal arteries were dissected and conserved for *in vitro* isolated vascular ring perfusion tests. In an organ bath tests were performed using increasing concentrations of serotonin (30 nmol L⁻¹ - 30 μmol L⁻¹). Several serotonin induced contraction cycles were performed in the presence of pre- and post-synaptic blockers (ondansetron, ketanserin, indomethacin). The same tests were performed on popliteal vascular rings from a healthy control who underwent a posttraumatic upper leg amputation.

Results: A tenfold higher serotonin concentration was needed to start contraction in carcinoid rings compared to control rings. Contractions in carcinoid rings were predominately mediated by 5-HT_{2A} receptors, whereas in normal popliteal artery there were also other functional serotonin receptors besides the 5-HT_{2A} subtype. In the normal popliteal artery serotonin vasoconstrictor control is supported by a pre-synaptic rescue mechanism, becoming active in case of blockade of smooth muscle 5-HT_{2A} receptor by ketanserin. This mechanism might be activated by serotonin stimulation and is probably mediated via contractile cyclooxygenase-derived prostanoids. Such a rescue mechanism by pre-synaptic activation appeared to be absent in carcinoid artery rings.

Conclusion: *In vitro* midgut carcinoid vascular rings are characterized by loss of pre-synaptic serotonin vasomotor control and non-responsiveness to low serotonin concentrations.

Introduction

A midgut carcinoid is a neuroendocrine tumor that can produce several metabolic active substances, including serotonin, prostaglandins (PGEs) and catecholamines.^{1,2} Vasomotor instability in patients with metastatic carcinoid disease is associated with hot flushes, hypotension or hypertension. Morphological changes can occur in the vessel wall (i.e. vascular elastosis) with thickening of the intima-media complex, resulting in reduced compliance of the vessel. This is observed, predominantly in mesenteric vessels, in the proximity of a carcinoid tumor mass. A well known distant effect of serotonin is carcinoid fibrotic heart disease.^{3,4}

Carcinoid disease is often associated with high serum levels of serotonin and the symptoms mentioned are at least partly considered to be due to serotonin.^{3,5} Elevated circulating serotonin levels in carcinoid patients might cause the display of impaired responses in the vessel wall. Pharmacologically consistent with long-term exposure to high serotonin levels, a downregulation of serotonin (5-HT)_{2A} receptors has been reported in platelets. Platelets form the serotonin reservoir of patients with carcinoid tumors.⁶ Apart from direct effects of serotonin on vascular tone also an indirect effect via so-called contractile PGEs might be involved.⁷ Highly increased plasma levels of contractile PGEs, particularly PGE₂, have been observed in carcinoid patients.⁸⁻¹⁰

So far, pharmacological studies on isolated arteries from carcinoid patients are not available. Normally serotonin causes at the vascular level constriction via activation of 5-HT_{2A} receptors present on the smooth muscle cells and/or activation of the 5-HT₃ receptor subtype present on post-ganglionic sympathetic neurons, representing an intrinsic transmitter-gated ion channel.¹¹ Dynamic responses to serotonin receptor stimulation within the vascular wall can be elegantly demonstrated using isolated vascular ring perfusions *in vitro*.^{12,13} The role of vascular serotonin receptors in carcinoid disease could be elucidated by analyzing the pre-synaptic inhibition on serotonin-induced contraction as well as the involvement of 5-HT_{2A} receptors in these responses and the role of contractile PGEs.

Therefore we evaluated in the present study the functional alterations in serotonin responses in isolated popliteal artery preparations of a carcinoid patient and a normal popliteal artery.

Methods

Patients

A 60-year-old patient with spina bifida and a metastatic midgut carcinoid was admitted with ischemia and necrosis of both underdeveloped and short legs (see figure 1). On admission both femoral and popliteal arteries were patent, and there was no apparent major arterial obstruction. It was hypothesized that a vasoconstriction of the arteries by circulating amines had resulted in recent ischemia. After 3 days the demarcation revealed irreversible ischemia necessitating an amputations. Immediately thereafter the popliteal arteries were dissected and collected in saline on ice and sent to the laboratory for *in vitro* vascular studies.



Figure 1: Both feet of the carcinoid patient at the time of admission with the peripheral necrosis.

A 57-year-old patient underwent an above knee amputation because of a non-union due to a recurrent osteomyelitis following an osteosynthesis of a comminutive femoral fracture one year earlier. The lower leg was unaffected. The popliteal artery was immediately dissected and collected in saline on ice, and transferred to the laboratory for *in vitro* vascular testing.

Preparation for in vitro studies with isolated popliteal artery rings

The arteries were cleaned of surrounding tissues and cut into several rings (2 mm). Rings were mounted in 15 ml organ baths, containing a buffer solution (Krebs) (mmol L^{-1}): NaCl (120.4), KCl (5.9), CaCl_2 (2.5), MgCl_2 (1.2), NaH_2PO_4 (1.2), glucose (11.5), NaHCO_3 (25.0). The medium was continuously aerated with 95% O_2 - 5% CO_2 and kept at 37 °C. The rings were connected to an isotonic displacement transducer by 5-0 braided, uncoated polyester sutures, where they received a preload of 1.4 g. The isotonic transducer, the recording system, and the software were custom made and calibrated at the University of Groningen, the Netherlands.

Artery rings were allowed to equilibrate for 60 min during which regular washing periods were performed. Rings were primed and checked for viability by repeated stimulation (three times) with 60 mmol L⁻¹ KCl and intermediate washing and stabilization periods. The third response to KCl was referred to as the 100% referral maximum contraction amplitude for each ring and all other contractile responses were expressed as a percentage of this response to further reduce inter-ring variability.

Experimental protocol for contractile responses to serotonin

Parallel rings were simultaneously studied during three consecutive series of measurements for contractile responses to increasing concentrations serotonin (30 nmol L⁻¹ - 30 μmol L⁻¹ bath-concentrations) in each series, and in the selective absence and presence of various compounds known to interfere with receptor signal transduction processes. In the first series of measurements, parallel rings were studied for serotonin responses under control conditions and under conditions of pre-synaptic inhibition. In the second and third series of measurement, the additional involvement of serotonin receptors and cyclooxygenase-derived PGEs was studied.

To create these conditions, we used vehicle as a control, 10 μmol L⁻¹ ketanserin to inhibit 5-HT_{2A} receptors¹⁴, 10 μmol L⁻¹ indomethacin to inhibit cyclooxygenase-derived PGEs⁷, and 1 μmol L⁻¹ ondansetron to obtain serotonin receptor mediated pre-synaptic inhibition.¹⁵ To obtain general pre-synaptic inhibition general a combination of ondansetron with 1 μmol L⁻¹ tetrodotoxin (an inhibitor of voltage sensitive Na⁺ channels¹⁶ and 300 μmol L⁻¹ suramin (a blocker of pre-synaptic P₂-purinergic receptors) was used.¹⁷ Rings were pre-incubated with the appropriate inhibitors for at least 30 min before stimulation with serotonin. Observations of serotonin contractility under certain conditions were obtained from 3-6 rings for each condition.

Thickening of the intima media complex causing a diminished compliance of the vessel wall, a well-known symptom in carcinoid patients, may interfere with contraction amplitudes. To correct for this and to reduce inter-ring variability, we expressed receptor-mediated vasoconstrictor responses to serotonin as percentage of maximal constriction with KCl.

Drugs used

The following chemicals and drugs were used: serotonin, indomethacin, ketanserine, suramin and tetrodotoxin (all from Sigma-Aldrich Chemie, Zwijndrecht, The Netherlands) ondansetron (Glaxo Smith Kline BV, Zeist, The Netherlands). Stock solutions were prepared of indomethacin (10 mmol L^{-1} , in ethanol), ketanserine (10 mmol L^{-1} , in H_2O), tetrodotoxin (1 mmol L^{-1} , in H_2O), suramin (300 mmol L^{-1} , H_2O), and ondansetron (1 mmol L^{-1} , H_2O), stored at -20°C and diluted in Krebs solution to reach final concentrations. Serotonin was prepared in H_2O (100 mmol L^{-1}), and further dilutions were performed directly in Krebs solution. Compounds for the Krebs buffer solution were all obtained from Merck (Darmstadt, Germany).

Calculations and statistical analysis

All responses of individual rings to serotonin were expressed as a percentage of the maximum contraction-response to 60 mmol L^{-1} KCl, and used for calculations and graphic representations. Maximal effect (E_{max}) and the effective concentration producing 50% of the maximal effect, expressed as negative logarithm (pEC_{50}), were obtained from the individual concentration-response curves. Observations of serotonin contractility under a specific condition were obtained from 3-6 rings. Comparison between percentual responses was made using Student's t-test. Data are expressed as mean \pm standard error of the mean (SEM). P-values <0.05 were considered significant.

Results

Receptor-independent contraction induced by a high concentration of KCl (60 mM), resulting in maximal contraction in popliteal artery rings was 40% lower in rings from the carcinoid patient ($593 \pm 73 \mu\text{m}$ versus 950 ± 68 displacement, $p < 0.001$). Serotonin induced, concentration-dependent contractions in popliteal artery rings were obtained from the control patient and the carcinoid patient (Figure 2A, B). Maximal contraction (E_{max}) was similar; in carcinoid rings: $51.8 \pm 8.7 \%$ and in control rings $52.2 \pm 7.5 \%$ of the maximal contraction as induced by 60 mM KCl. Also pEC_{50} values were not different (carcinoid rings: 5.9 ± 0.1 ; control rings 5.8 ± 0.1), however the threshold of contraction was at a much higher serotonin concentration for carcinoid-derived rings $1 \mu\text{M}$ than $0.1 \mu\text{M}$ for control rings. In the presence of pre-synaptic inhibitors

similar results were obtained. Treatment with ondansetron alone, to block pre-synaptic serotonin receptors, was as ineffective as when given in combination with other inhibitors of post-ganglionic synaptic transmission, tetrodotoxin and suramin (data not shown). Indomethacin as inhibitor of cyclooxygenase-derived prostanoids synthesis, only slightly changed contraction in carcinoid rings (E_{\max} : 26.2 ± 5.9 %; pEC_{50} : 5.7 ± 0.1 , Figure 2A, $p=n.s.$) and control rings (E_{\max} : 34.6 ± 10.7 %; pEC_{50} : 5.8 ± 0.1). In contrast, the 5-HT_{2A} receptor blocker ketanserin totally abolished contractions in carcinoid vascular rings with an E_{\max} : -5.6 ± 0.1 % and after presynaptic inhibition -8.3 ± 2.6 %; $p<0.05$. In control rings ketanserin decreased contractions to 37.0 ± 0.4 % with pEC_{50} : 6.5 ± 0.1 ($p<0.05$) and in the presence of presynaptic inhibition even further (E_{\max} : 13.4 ± 5.6 %; pEC_{50} : 6.0 ± 0.1 ; $p<0.05$). The combination of ketanserin and indomethacin in control rings slightly decreased contraction in the absence and presence of presynaptic inhibitors to E_{\max} : 10.0 ± 3.2 % and -0.5 ± 3.9 %, respectively (Figure 2E), but totally blocked contractions in carcinoid rings.

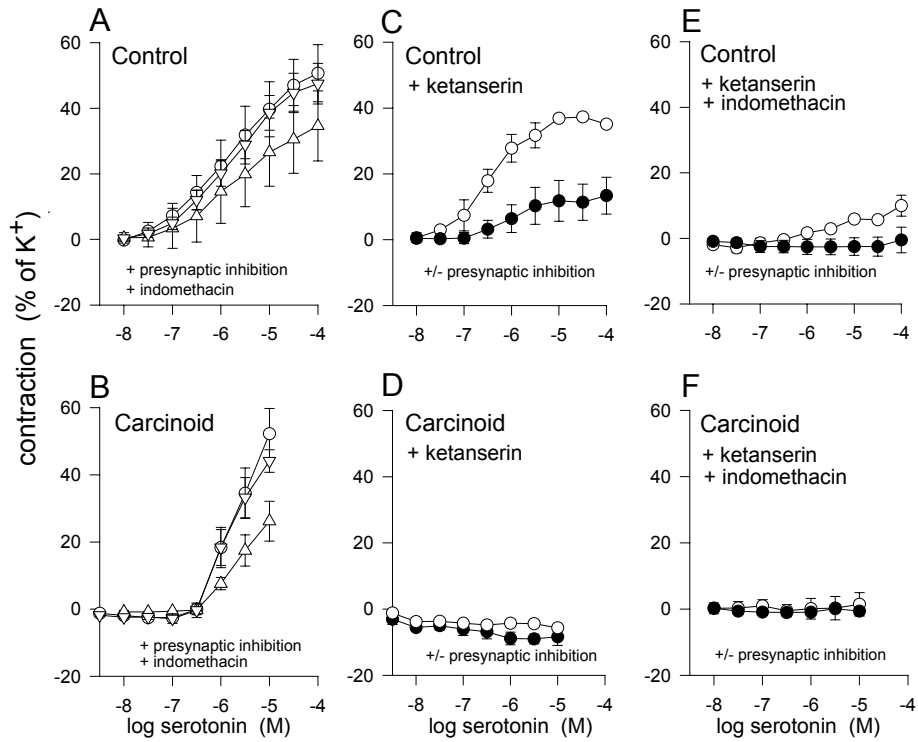


Figure 2:

Concentration-response curves of vascular rings prepared from the control patient (A, C, E) and carcinoid patient (B, D, F). Various conditions were applied: In panel A and B “control” (○), under pre-synaptic inhibition (∇), and under inhibition of prostaglandin synthesis by indomethacin (△). In panel C and D in the presence of the 5-HT_{2A} inhibitor ketanserin, “control” (○) and under pre-synaptic inhibition (●). In panel E and F in the presence of 5-HT_{2A} receptor blockade and inhibition of prostaglandin synthesis, “control” (○) and under pre-synaptic inhibition (●). Contraction was defined as % 60 mM KCl induced contraction and presented as mean ± SEM.

Discussion

This is the first study, on pharmacologically characterized vascular function of isolated arteries obtained from a patient with midgut carcinoid. Some striking differences became apparent in these arteries compared to arteries from a control patient. Carcinoid-derived popliteal artery rings were less responsive to the receptor-independent contractile agent KCl than control popliteal artery rings (0.6-fold). This decrease in contractility is probably due to structural changes in the vessel wall as observed in carcinoid patients.^{4,18} Interestingly, a similar reduction of contractility due to structural changes in the vessel wall has been reported for popliteal artery rings obtained from patients with peripheral occlusive arteriosclerosis.¹⁹

When normalized for the maximal KCl contraction, receptor-induced contraction to serotonin appeared to be very similar for carcinoid rings and control. The presence of pre-synaptic inhibitors did not affect the responses to serotonin. This suggests that modulation of muscle contraction by activation of post-ganglionic neurons is not influenced by pre-synaptic factors. However, when the rings were incubated with the 5-HT_{2A} blocker ketanserin, contractions in carcinoid rings were totally blocked while control rings were still able to contract (figure 2A versus 2B). This contraction must be mediated via contractile PGEs and mainly involves pre-synaptic activation via pre-synaptic 5-HT₃ receptors in view of the results obtained with ondansetron and the PGE blocker indomethacin experiments (figure 2 C and E). This rescue mechanism is absent in carcinoid vascular rings suggesting loss of pre-synaptic control in these rings. A scheme depicting the hypothesized explanations of these findings is shown in figure 3. The mechanism by which this occurs remains to be solved. It could resemble the observations in tracheal ring preparations of the guinea-pig. In this previous study, a chain of events leads from receptor activation to production of contractile PGEs and subsequent smooth muscle contraction via prostanoid receptors.²⁰ PGE synthesis and secretion was induced by activation of Ca²⁺-dependent cytosolic phospholipase-A2, generating arachidonic acid, the substrate for cyclooxygenase.⁷ As mentioned, serotonin receptor activation results in Ca²⁺ influx, which might be well capable of starting similar events in the arteries tested. Functional downregulation of the pre-synaptic serotonin receptor subtype in carcinoid rings, due to long-term exposure to high serotonin concentrations *in vivo*, might be responsible for the

disappearance of the rescue mechanism in carcinoid patients. A general neuropathy as cause is less likely, since the effect observed is already detectable by solely blocking serotonin receptors by ondansetron. We studied the baroreflex sensitivity in carcinoid patients with elevated platelet serotonin and showed a reduced baroreflex sensitivity compared to sex and age matched controls.²¹ Reason for this may be the loss of the fine-tuning of the sympathetic presynaptic control.

A ten-fold high serotonin was required to induce contractions in carcinoid vascular rings compared to control rings (Figure 2B). This is consistent with downregulation of 5-HT_{2A} receptors as observed in platelets of patients with carcinoid tumors.⁶

In conclusion, this is the first report to demonstrate that in the popliteal artery of a carcinoid patient the constriction of an isolated vascular ring was predominately mediated by 5-HT_{2A} receptors, whereas in normal popliteal artery there are also other functional serotonin receptors. Our results suggest, that in normal popliteal artery serotonin vasomotor control is supported by a pre-synaptic rescue mechanism, becoming apparent after blocking of the smooth muscle 5-HT_{2A} receptor. This mechanism might be activated by serotonin stimulation and can be blocked by indomethacin indicating a role for contractile cyclooxygenase-derived prostanoids. Such a rescue mechanism seems to be absent in carcinoid arteries.

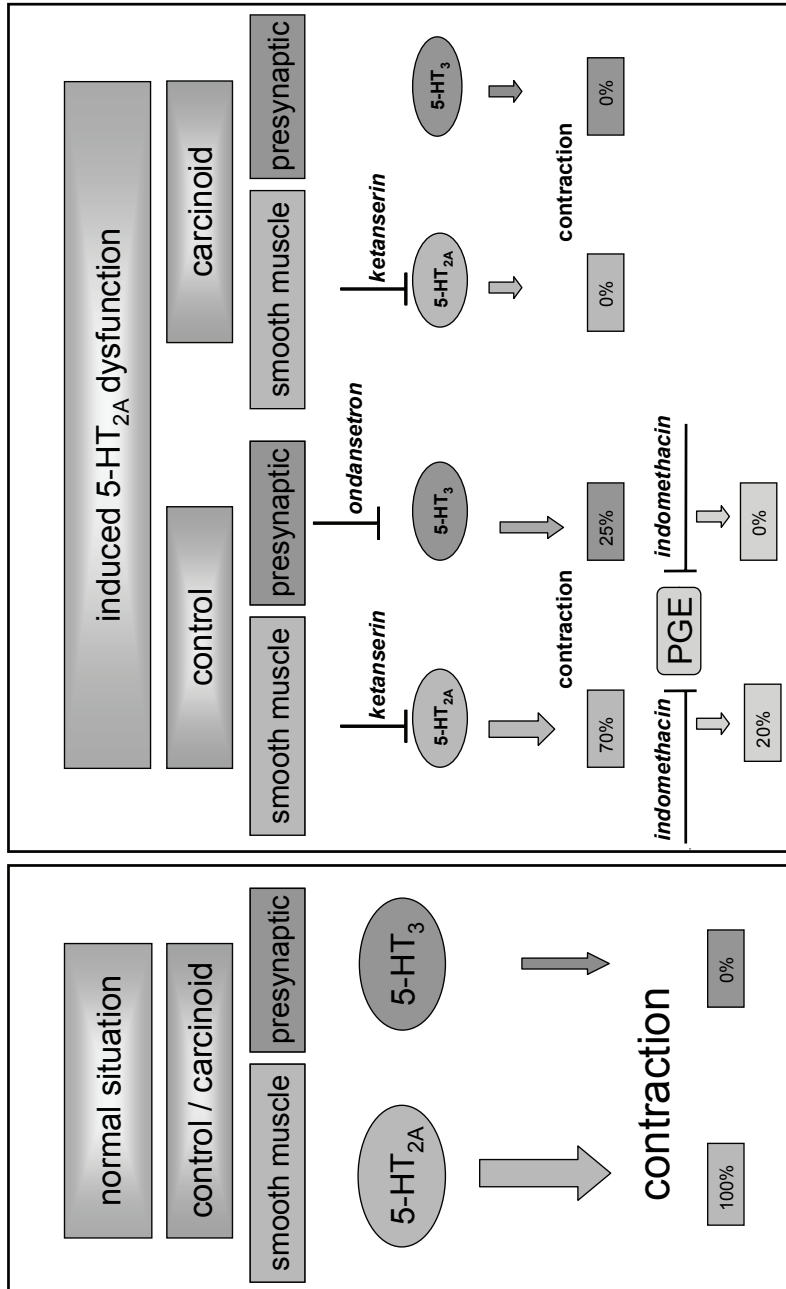


Figure 3: Schematic representation depicting the observed differences between carcinoid and control vessels. Two situations are presented, in the left panel the 'normal situation' and in the right panel the 'dysfunctional' by blocking with ketanserin. In the normal situation in carcinoid as well as in control rings, serotonin-induced contraction is mainly mediated by 5-HT_{2A} receptors on the smooth muscle cells and not by pre-synaptic stimulation via 5-HT₃ receptors. Blocking the 5-HT_{2A} receptor by ketanserin (right panel) abolishes the contraction in carcinoid rings whereas control rings whereas control rings can rescue 70% of the contraction, presumably via contractile PGE₂s as indomethacin can block this contraction to only 20%. After 5-HT₃ receptor inhibition by ondansetron 'rescued' contraction has dropped to 25%. In this experimental setting carcinoid derived vessels did not possess such rescue mechanism.

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