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ORIGINAL PAPER

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Plasma levels of cyclic guanosine-3′,5′-monophosphate in the cavernous and systemic blood of healthy males during different functional conditions of the penis

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Abstract The relaxation of cavernous arterial and trabecular smooth muscle is dependent upon the stimulation of guanylyl cyclase activity by nitric oxide (NO), which is released from nerve terminals and endothelial cells within the cavernous tissue, and the subsequent accumulation of cyclic guanosine-3′,5′-monophosphate (cGMP) in the intracellular space. The present study was undertaken to determine whether or not plasma levels of cGMP in the systemic and cavernous blood of healthy male subjects change from penile flaccidity to tumescence, rigidity and detumescence. Fifteen adult healthy males were exposed to visual and tactile erotic stimuli to elicit penile tumescence and rigidity. Whole blood was simultaneously aspirated from the corpus cavernosum and the cubital vein in the respective penile stages, and cGMP was determined in plasma aliquots by means of a radioimmunoassay. Mean systemic and cavernous plasma levels of cGMP in the blood samples obtained from the healthy volunteers ranged from 1.2–1.7 pmol/ml. cGMP levels in the systemic circulation and in the cavernous blood did not change during developing erection, rigidity and detumescence. No significant differences were found between cGMP plasma levels in the systemic and cavernous blood in the different penile stages. Our results may reflect the fact that the stimulation of NO production in healthy males during sexual arousal and developing penile erection either does not yield substantial quantities of cGMP or that the rate of cGMP-extrusion from cavernous smooth muscle cells into the extracellular space accounts only for a minor fraction of plasma cGMP. Moreover, basal levels of cGMP in the blood flushing the lacunar spaces of the cavernous body in the state of developing erection may conceal any local release of cGMP that may occur within the penile erectile tissue. Thus, we conclude that the quantification of cGMP is of no use in the evaluation of the physiologic mechanisms of penile erection in vivo.

Keywords Nitric oxide · cGMP · Corpus cavernosum · Physiological mechanisms · Penile erection

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Introduction

Penile erection results from a complex interaction of neuronal, hormonal, vascular and myogenic events involving the action of several neurotransmitters and vasoactive agents [2,1]. Relaxation of penile vascular and trabecular smooth muscle is the crucial event in the initiation and maintenance of male penile erection. Upon sexual stimulation, this event is brought about by the release of nitric oxide (NO) from nonadrenergic-noncholinergic nerve terminals within the cavernous tissue, as well as from the endothelial layers lining the cavernous spaces and the penile arteries [9,22]. NO penetrates the trabecular smooth muscle cells and promotes the synthesis of guanosine-3′5′-cyclic monophosphate (cGMP) by the soluble form of guanylyl cyclase. This increase in cGMP triggers a signal transduction cascade, which encompasses the activation of cyclic nucleotide-dependent protein kinases, subsequent phosphorylation of the actin-myosin system and Ca^{2+} channels located in the outer-cell membrane and in the membrane of the sarcoplasmatic reticulum. This signal transduction cascade leads to a reduction in free cytosolic Ca^{2+} and, finally, to smooth muscle relaxation [14, 16, 20]. The discovery of NO and cGMP as important effectors in penile smooth muscle relaxation and, thus, erectile function, has led to the development of certain drugs that are able to enhance this pathway through the elevation of intracellular cGMP levels. Among these

agents are nitric oxide donors, such as nitroglycerine [8] and linsidomine (SIN-1) [27], and compounds that inhibit the activity of the cGMP-hydrolyzing phosphodiesterase type 5 (PDE5), such as pentoxifylline, sildenafil, IC 351 and BAY 38–9456 [13, 5, 6, 18, 23].

Recent papers presented serious efforts to evaluate whether plasma levels of NO metabolites nitrite and nitrate (NO_2^-/NO_3^-) are elevated in the systemic and/or cavernous blood of male subjects during penile tumescence and rigidity in response to sexual arousal [3,17]. Despite the application of sophisticated methods to detect NO_2^- and NO_3^-, the authors were unable to demonstrate that in healthy males the systemic or local concentrations of these NO metabolites increased with developing penile erection. Moreover, the comparison of healthy males and patients presenting with erectile dysfunction revealed no differences in the systemic and cavernous release of NO [3]. In fact, the determination of NO metabolites, even with advanced detection methods, might be impaired by the modification of the physicochemical properties of NO_2^- and NO_3^- through derivatization or interaction with native circulating substances, such as albumin, haem and reduced thiols [29]. Thus, alternative approaches are required to determine the stimulation of the NO–cGMP cascade in male subjects in response to sexual arousal. One possible way to pursue this hypothesis might be to determine the levels of the second messenger cGMP in the cavernous and systemic blood of males prior to and during developing and sustained penile erection. There are two major pathways to eliminate cGMP from the cell: degradation by phosphodiesterase enzymes, which cleave the 3′,5′ ribose-phosphate bound of the molecule, or extrusion from the cytoplasma into the extracellular space by an ATP-driven transporter protein, the so-called multidrug resistance-associated protein 5 (MRP5). MRPs belong to a family of ATP-binding cassette transporter proteins, which confer resistance to some anticancer drugs and efflux nucleotide, glutathione and glucuronate conjugates from the cell [10, 30, 31]. Therefore, since cGMP appears in the circulation before it is finally excreted with the urine from the body, it is possible to determine whether plasma levels of cGMP change in response to metabolic or pharmcological stimuli or general pathological conditions.

The present study was performed to determine plasma levels of cGMP in systemic and cavernous blood samples withdrawn from healthy males during different functional conditions of the penis (flaccidity, tumescence, rigidity, detumescence).

Material and Methods

Blood withdrawal

Fifteen healthy adult males (same volunteers as in the previous protocol described in Becker et al. [3]) aged 19 to 44 years (mean age 26 years) with normal erectile function were empanelled into the study after written informed consent was obtained. The study was approved by the local Ethics Committee of the Hannover Medical School. Each subject completed a comprehensive questionnaire on medical history and sexual behaviour. Subjects were placed in a supine position with the upper part of the body in a 20°–40° upright position. A 20-gauge (G) intravenous indwelling cannula (Vasofix Braunüle, B. Braun AG, Melsungen, Germany) was inserted into the left cubital vein, and a 19G butterfly needle (Abbott Laboratories Ltd., Sligo, Ireland) was inserted into the left corpus cavernosum. Following a resting period, blood was drawn from the cubital vein and the corpus cavernosum in the phase of the flaccid penis. Volunteers were then exposed to sexually explicit video movies and self-stimulation of the glans penis. During the penile phase of tumescence, blood was aspirated simultaneously via the butterfly needle from the corpus cavernosum and the cubital vein. Blood was drawn again in the state of penile rigidity. After the volunteers had developed penile erection, sexual stimulation was terminated, and blood was again simultaneously drawn in the phase of detumescence. For blood collection S-Monovetten (Sarstedt, Nümbrecht, Germany) were used containing 1.6 mg (K^+) ethylenediaminetetraacetic acid (EDTA)/ml whole blood. Tubes were put on ice immediately after blood withdrawal. Blood samples were centrifuged at 4°C for 10 min at 3,000 rpm (Cryofuge 5000, Heraeus-Christ, Osterode, Germany), and plasma was then aspirated and stored at –80°C until analysis.

Determination of cGMP

Plasma aliquots (200 µl) were actylated by the addition of 7 µl of a mixture consisting of two parts of triethylamine and one part of acetic anhydride. 100 µl of a suspension containing rabbit antibody against cGMP (Dilution: 1:200,000 in 0.15% gamma globulin solution) and 50 µl of a radionuclide solution (^{125}[J]cGMP in 50 mM sodium acetate buffer, pH 6.0, 5,000–7,000 cpm) were added to a total of 50 µl of the final sample, and the mixture was allowed to incubate at 4°C for at least 12 h. Precipitation of antibody-antigen complexes was carried out by the addition of 100 µl of a 0.8% gamma-globulin solution (in sodium acetate buffer) and 750 µl of a 15% polyethylene glycol solution (in 10 mM TRIS/HCl buffer, pH 7.4), followed by a 30-min incubation period. Following centrifugation (20 min at 3,000 rpm), the supernatant was discarded, and the activity in the remaining pellet was measured using a gamma counter (WALLAC Wizzard Automatic Gamma Counter, Turku, Finland). Each sample was assayed in duplicate. A standard curve of acetylated cGMP in the concentration range of 20 nM/l–0.08 nM/l (20, 10, 5.0, 2.5, 1.25, 0.63, 0.3, 0.16 und 0.08 nM/l) served as a reference. Nonspecific binding was calculated from a sample containing 50 µl acetylated sodium acetate buffer, 100 µl of 0.15% gamma-globulin solution and 50 µl of radionuclide solution.

For comparison of cGMP levels in the systemic and penile blood samples, the Student's *t*-test for paired samples of the SPSS 7.5 for Windows (SPSS Inc. Chigaco, Ill., USA) was used.

Chemicals

Triethylamine, acetic anhydride and polyethylene glycol were obtained from Merck KGa (Darmstadt, Germany), TRIS from Sigma Chemical Company (St. Louis, Mo., USA), γ-globulin was from SERVA Feinchemikalien GmbH, Heidelberg, Germany. ^{125}[J]cGMP was purchased from Amersham-Pharmacia Biotech Europe GmbH (Freiburg, Germany). Antibodies raised in rabbits against cGMP were generously provided by the Lower Saxony Institute of Peptide Research GmbH (Hannover, Germany).

Results

One subject terminated his participation in the study because of persisting penile pain after insertion of the butterfly needle into the corpus cavernosum. Blood

withdrawal from the corpora cavernosa in the flaccid state of the penis was facilitated in 11 out of the remaining 14 volunteers. All subjects developed penile tumescence and rigidity. Blood sampling from the cavernous body in the phase of penile detumescence took place in 13 out of 14 volunteers.

Determination of cGMP revealed no differences before and during penile tumescence and rigidity either in the cavernous or the systemic blood cavities taken from the volunteers. Mean cGMP levels were registered ranging from 1.2–1.7 pmol/ml. No significant differences in cGMP-levels measured in the systemic and cavernous blood of the volunteers were found. The results are summarized in Table 1 and Fig. 1.

Discussion

Penile erection strictly depends upon the balance of vascular and trabecular smooth muscle constriction and relaxation. Up until now, it has been fairly well demonstrated that relaxation of cavernous smooth muscle is under the control of a variety of endothelial-derived relaxing factors among which NO has been identified as the most important mediator of penile erection [2,1]. Since cGMP mediates most of the effects of NO, and thus plays an important role in smooth muscle relaxation, cGMP has emerged as a major focus in cavernous signal transduction research. Nevertheless, to date, there is no evidence that systemic or local levels of NO in males increase with sexual arousal, penile tumescence or rigidity. Cellular levels of cGMP are determined by the ratio of the synthesis by guanylyl cyclases and the rate of elimination within or from the cell. It has been shown that the formation of cGMP in vascular endothelial and smooth muscle cells in response to pharmacological stimuli is accompanied by the secretion of this molecule from the cell, and the appearance of cyclic nucleotides in blood and urine has been known for decades [7,21]. Thus, it seems rational to evaluate plasma levels of cGMP in the systemic and cavernous blood of healthy males during different penile conditions.

The present study revealed no appreciable changes in cGMP-levels in the systemic and cavernous blood before, during and after penile erection. This is well in accordance with earlier findings made by Moriel and coworkers that an increase in NO release cannot be detected in the systemic and cavernous blood of healthy males during penile tumescence and erection [17]. These results have recently been confirmed by our group using a comparable study set-up and more advanced methods (gas chromatography – mass spectrometry) for the detection of NO metabolites NO_2^- and NO_3^- in biological fluids [3].

Several in vivo studies, which investigated a maximum number of $n=6$ to $n=59$ volunteers, evaluated possible correlations between plasma levels of cGMP and pharmacological stimuli (infusion or inhalation of NO, NO-donors or L-arginine) or particular physiological conditions of the human body (pregnancy, female preeclampsia, diabetes, hypertension, liver failure) [4, 11, 12, 15, 19, 24, 25, 28, 32]. The mean basal cGMP plasma levels presented in these papers range from 1.77 to 2.5 pmol/ml, which is a little higher than the mean concentrations we determined in our study (systemic blood: 1.53–1.72 pmol/ml; cavernous blood: 1.23–1.73 pmol/ml).

Lopez-Jaramillo et al. and Szymanski et al. demonstrated an increase in plasma levels of cGMP in the late phase of pregnancy and in women at the time of spontaneous uterus contractions, respectively, and Schneider et al. found that plasma cGMP was significantly increased in women with preeclampsia symptoms [15, 28,25]. They also reported higher cGMP concentrations in the plasma of patients with fulminant liver failure compared with those patients who underwent curative abdominal surgery [24]. Zwissler et al. in their study described an increase in plasma cGMP in response to

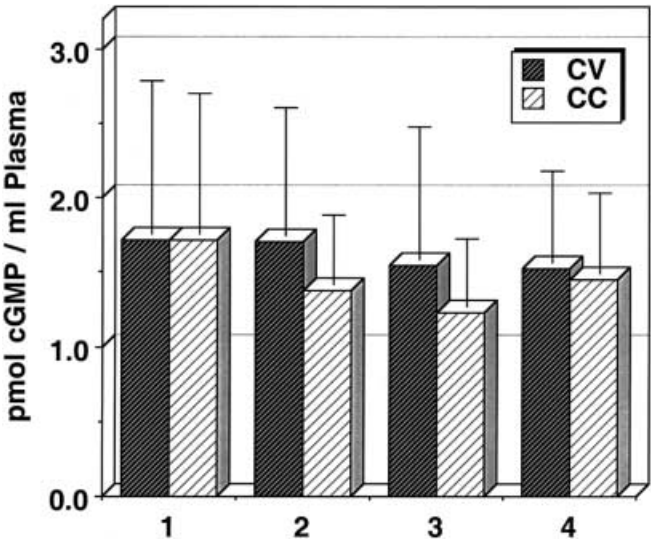

Fig. 1 Plasma levels (pmol/ml) of guanosine-3′,5′-cyclic monophosphate in systemic and cavernous blood samples obtained from healthy males at different conditions of the penile erectile tissue (*1* flaccidity, *2* tumescence, *3* rigidity, *4* detumescence). *CV* cubital vein, *CC* corpus cavernosum. See Table 1 for details

Table 1 Plasma levels of cGMP (pmol/ml) determined in systemic and cavernous blood samples obtained from healthy male volunteers at different functional conditions of the penis

Penile Condition	Blood Source	Blood Withdrawals	cGMP (pmol/ml)
Flaccidity	Cubital vein	14	1.72 ± 1.02
	Penis	11	1.72 ± 0.94
Tumescence	Cubital vein	14	1.71 ± 0.85
	Penis	14	1.38 ± 0.46
Rigidity	Cubital vein	14	1.53 ± 0.89
	Penis	14	1.23 ± 0.46
Detumescence	Cubital vein	14	1.53 ± 0.61
	Penis	13	1.45 ± 0.53

inhaled NO in patients with acute lung injuries [32], and Bode-Böger et al. demonstrated that infusion of the amino acid L-arginine, the native substrate of cellular nitric oxide synthase enzymes, corresponded with an elevation of plasma NO_2^- and cGMP in healthy male subjects [4].

In contrast, there are some reports that indicate that a stimulation of the NO-cascade in vivo by means of pharmacological agents or metabolic changes is not necessarily paralleled by an obvious increase in cGMP concentrations in body fluids. In healthy volunteers, Karrenbrock and coworkers did not detect elevation of plasma cGMP levels in response to the intravenous administration of the NO-donor 3-morpholino-sydnonimine (SIN 1) [11], and Kohno et al. reported a correlation between systolic, diastolic and mean blood pressure and NO, but not cGMP-plasma levels after long-term treatment of hypertensive patients with angiotensin-converting enzyme inhibitors [12]. In a study regarding alterations in the NO/cGMP pathway in nondiabetic siblings of patients with type 2 diabetes, Piatti and coworkers reported that NO-levels, evaluated by the measurement of NO_2^- and NO_3^-, were significantly higher, whereas cGMP-levels were lower in the blood of subjects with a family history of type 2 diabetes [19]. Thus, one can speculate that the local stimulation of NO production in males during sexual arousal and developing penile erection does not yield substantial quantities of cGMP or that the rate of cGMP-extrusion from cavernous smooth muscle cells by the activity of MRP5 membrane proteins account only for a minor fraction of plasma cGMP.

When discussing the results of previous studies, as well as from the present experimental protocol, it should be taken into consideration that changes in penile hemodynamics, characterised by an increase in systemic arterial inflow into the cavernous compartment during tumescence and rigidity, may mask any release of NO and cGMP that might occur within the penile erectile tissue [26]. Then, small changes in the release of cGMP in the cavernous compartment in the phases of penile tumescence and rigidity might be masked by the basal levels of this molecule in the systemic arterial blood that flushes the corpus cavernosum meshwork during these particular penile stages. The present study demonstrates that the determination of cGMP plasma levels does not provide accurate evidence for the release of NO within the human corpus cavernosum during tumescence and rigidity. Since studies have demonstrated that there are no differences in systemic and cavernous plasma concentrations of NO_2^- and NO_3^- in blood cavities taken during different functional states of the penile erectile tissue from healthy subjects and patients with erectile dysfunction of both organogenic and psychogenic etiology [3], it seems extremely unlikely that the determination of systemic and cavernous cGMP plasma levels in a group of patients with erectile dysfunction will unravel any differences in comparison to healthy males. In conclusion, the quantification of cGMP in the blood seems to be of no use in the experimental workup of the in vivo mechanisms of penile erection and the pathophysiology of male erectile dysfunction.

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