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Genital Blood Flow in Male Rats Subjected to Stress Stimuli

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Summary. A common opinion among physicians is that some symptoms from the prostate gland are stress-dependent. However, no experimental support for this view has been presented. In the present study blood perfusion of the major genital organs of male rats were measured after they had been subjected to experimental "short- and long-term" stress stimuli. The blood-flow measurements were made by means of a radioactive microsphere technique. The used experimental "short-term" stress reduced the prostatic blood flow by approximately 50 per cent. A decreased blood flow and a concomitant atrophy, indicating that the reduction in blood flow has been in force over a prolonged period of time, were noticed in all examined genital organs of the rats subjected to prolonged stress stimuli. The decreases in blood flow and weight were most pronounced in the prostate gland.

Key words: Atrophy, Blood flow, Experimental study, Prostatic gland, Radioactive microsphere technique, Experimental stress.

Introduction

It has been suggested that the prostate gland is sensitive to stress factors, and physicians commonly hold the view that stress stimuli aggravate the symptoms of prostatitis [1, 11–13]. No experimental results supporting this opinion have been presented.

The regulation of blood perfusion of the male genital organs has not been fully clarified. In a previous study [4] we found that the radioactive microsphere method, first described by Rudolph and Heymann in 1967 [17], is precise and is suitable for experimental blood flow measurements on rats. We found that, under standard conditions, the blood flow in the prostate gland varies from one individual to another. The variations of the prostatic blood flow were not correlated to corresponding variations in the other male urogenital organs studied. On the contrary, we noticed that, in the same individual, a low blood perfusion rate in the kidneys was accompanied by correspondingly low rates in the testicles and seminal vesicles.

The endocrine and exocrine activities of a gland are considered to be related to its blood perfusion [2].

Stress caused by mental and/or physical factors is accompanied by an increased catecholamines measurable in the blood [10]. Catecholamines reduce the blood flow in the testis [19, 20]. This suggests that these substances may have a corresponding effect directly or indirectly on the blood perfusion of the prostate gland. If so, the effects of stress stimuli would be a decreased blood perfusion of the prostate gland which subsequently could result in morphological and functional disturbances.

The purpose of the present investigation was to elucidate the influence of stress stimuli on blood perfusion of the prostatic gland.

Materials and Methods

Twenty-nine male Sprague-Dawley rats (bred by Anticimex, Sollentuna, Sweden), weighing approximately 350 g each, were used in the study.

The rats were kept at the Department under standarized conditions for a minimum of one week prior to the respective experiment in cages measuring 500 x 400 x 150 mm with four rats in each cage. They had free access to water and food. The animals were randomly allocated into two groups of 9 and 20 rats, respectively.

In the first group of nine rats, blood flow was determined by means of the radioactive microsphere technique, according to Rudolph and Heymann [17]. Carbonized microspheres (3M Tracer Microspheres^{R}, 3M Company, St. Paul, Minnesota, USA), diameter 15 ± 5 μm, labelled with strontium-85 or cerium-131, were used. The method and experimental handling of the animals during the measurement procedures have previously been described by us [4]. After being allowed 5 min rest after the first microsphere injection, the animals were subjected to an additional standardized surgical trauma, by means of a laporotomy procedure, considered as a stress stimulus. All the rats showed signs of pain at the laparotomy. As

Table 1. Schedule showing the "prolonged" stress stimuli – immobilisation, starvation, cooling and changed diurnal rhythm – to which each one of the 20 test male rats, in part II of the present study, was subjected to. Normal cages = Four rats in each cage, 500 x 400 x 150 mm. Small cages = Seven rats in each cage, 200 x 200 x 100 mm

Day number	Cages	Free access to food and water	Room temperature
1	normal	no	20 °C
2	normal	yes	4 °C
3	small	yes	20 °C
4	normal	yes	20 °C
5	normal	no	20 °C
6	normal	yes	4 °C
7	small	yes	20 °C
8	normal	yes	20 °C
9	normal	no	20 °C
10	normal	yes	4 °C

Table 2. Mean values (± SD) of the blood flow in the prostatic gland, seminal vesicles, testicles, kidneys, heart and cardiac output (C.O.) in nine male rats prior to (control values), and when submitted to, an acute surgical trauma (stress values) and their respective percentage deviations

Organ	Blood flow (ml/100 g/min)		
	No stress stimuli	Stress stimuli	Percentage deviation
Prostate	29 ± 12	14 ± 7	− 50
Sem. vesicles	25 ± 8	15 ± 6	− 30
Testicles	25 ± 8	16 ± 5	− 29
Kidneys	366 ± 130	194 ± 66	− 43
Heart	430 ± 246	444 ± 105	± 0
C.O.	35 ± 9	19 ± 5	− 44

soon as laparotomy was completed, a new injection of microspheres was performed in exactly the same way as the first. Microspheres labelled with strontium-85 and cerium-141 were injected alternately.

Immediately before each microsphere injection, an arterial blood sample was taken for blood glucose examination to check the condition of the animal.

The animals were instantly killed after the second injection by cutting through the aorta. The heart, kidneys, prostatic gland, seminal vesicles and testicles were sampled. The dissections of the urogenital organs were performed in accordance with previously described techniques [14]. At the dissection of the prostatic gland efforts were made to free both the ventral and dorsolateral lobes. After weighing the sampled specimens, the organs and the reference blood samples were analysed in a two channel gamma scintillation detector (Philips-Harshaw, Harshaw Chemical BV, Amsterdam, The Netherlands) connected to a counter (Model 54-22, Selektronic, Copenhagen, Denmark).

The regional blood flows (Q) were determined according to the equation:

$$Q = m \times Qs \times ms^{-1}$$

where m is tissue activity, Qs blood sampling rate and ms activity of the reference blood sample. Blood flow was expressed as ml x min^{-1} x 100 g tissue wet $weight^{-1}$.

The twenty male rats in the second group were randomly allocated to two groups, A and B, containing 10 animals each.

All rats in each of these two test groups (A, B) were subjected to standardized stress stimuli procedures for 10 days. These procedures included starvation, low surrounding temperature (4 °C), immobilization and a changed diurnal rhythm. The schedule is presented in Table 1.

After ten days the genital organ blood flow was determined in all the rats, as described above, but with the laparotomies omitted. All measurements were performed within one hour after the respective animal had completed its period of stress stimuli. Only one injection of microspheres was performed in each rat. Microspheres labelled with strontium-85 and cerium-141 were injected alternately. Immediately before the microspheres were injected, a blood sample was taken for blood glucose examination, in order to check the condition of the rat. The animals were instantly killed after the injection by cutting through the aorta. In group A, the kidneys, prostatic gland and seminal vesicles were sampled. In group B, the kidneys and testicles were sampled.

The recorded blood flow values were compared to those obtained in 14 previously tested rats not subjected to any corresponding experimental stress stimuli [4]. These later values have been handled as reference values in the present study.

Conventional statistical methods were used. Intra- and intergroup mean values were compared according to the Man-Whitney test for dependent and indpendent observations. Prior to the experiments, $p < 0.05$ was fixed as significant.

Results

I. In the nine male rats submitted to "short-term" stress stimuli produced by standardized analgesia and laparotomy (group I), we recorded:

- a significant ($p < 0.05$) reduction of blood flow in the prostatic gland – approximately 50 per cent (Table 2).
- a significant ($p < 0.05$) reduction of blood flow in the testicles and seminal vesicles (Table 2).
- a significant ($p < 0.05$) reduction of blood flow in the kidneys (Table 2).
- no significant change ($p > 0.05$) of blood flow in the heart (Table 2).
- a significantly ($p < 0.05$) reduced cardiac output (Table 2).
- no significant change ($p > 0.05$) in mean arterial blood pressure (Means ± SD: 89 ± 12 and 76 ± 9 mmHg, respectively).

The average blood glucose concentrations before and after laparotomy were 10.3 and 13.3 mmol/l respectively.

II. In 20 male rats submitted to standardized experimental stress stimuli for 10 days ("prolonged" stress stimuli), the following observations were made compared with the reference values obtained form 14 untreated rats, considered as controls:

a. • a significantly ($p < 0.05$) decreased body weight – approximately 20 per cent.

Table 3. Mean wet weights (± SD) of the prostate glands, testicles and seminal vesicles in 10 male rats submitted to standarized stress stimuli for 10 days and in 14 previously tested controls not submitted to corresponding experimental stress stimuli, and the means (± SD) of the relative wet weights of the organs compared to total body weight

Organ	Organ weight (g)		Organ weight/Body weight (per cent)	
	Test group prol stress st	Controls	Test group prol stress st	Controls
Prostate	0.26 ± 0.03	0.50 ± 0.07	0.10 ± 0.01	0.14 ± 0.02
Sem. vesicles	0.34 ± 0.08	0.52 ± 0.07	0.13 ± 0.03	0.14 ± 0.02
Testicles	3.10 ± 0.32	3.32 ± 0.44	1.10 ± 0.12	1.01 ± 0.14

Table 4. Mean blood flows (± SD) in the prostate gland, seminal vesicles, kidneys and cardiac output (C.O.), in 10 male rats subjected to experimental "prolonged" stress stimuli for 10 days, and the corresponding values in 14 previously tested male rats no subjected to corresponding experimental stress stimuli (controls). Mean values of paired organs are given

Organ	Blood flow (mg/100 g/min)	
	Test group prol stress stim	Controls
Prostate	14 ± 3	28 ± 9
Sem. vesicles	8 ± 6	24 ± 8
Kidneys	279 ± 59	352 ± 125
C.O.	27 ± 7	35 ± 7

Table 5. Mean blood flows (± SD) in the testicles and kidneys, and cardiac output (C.O.), in 10 male rats subjected to experimental "prolonged" stress stimuli for 10 days, and the corresponding values in 14 previously tested male rats not subjected to corresponding experimental stress stimuli (controls). Mean values of paired organs are given

Organ	Blood flow (ml/100 g/min)	
	Test group prol stress stim	Controls
Testicles	11 ± 5	22 ± 7
Kidneys	345 ± 141	352 ± 125
C.O.	30 ± 13	35 ± 7

- significantly ($p < 0.05$) decreased wet weights of the prostate glands, seminal vesicles and testicles (Table 3).
- a significantly ($p < 0.05$) decreased ratio prostate gland/total body weight – approximately 30 per cent (Table 3).
- no significant change ($p > 0.05$) in the ratio seminal vesicles respective testicles wet weight compared to the total body weight (Table 3).

b.
- a significantly ($p < 0.05$) decreased blood flow in the prostate gland – approximately 50 per cent (Table 4).
- significantly ($p < 0.05$) decreased blood flows in the seminal vesicles and testicles (Tables 4, 5).
- no significant change ($p > 0.05$) in kidney perfusion (Tables 4, 5).
- no significant change ($p > 0.05$) in cardiac output (Tables 4, 5).
- no significant change ($p > 0.05$) in mean arterial blood pressure (Means ± SD: 89 ± 10 and 92 ± 15 mmHg, respectively).

The mean concentrations of glucose in blood were 5.7 mmol/l in the test groups and 8.5 mmol/l in the controls.

Discussion

In the light of clinical experience, the prostate gland is considered a stress-sensitive organ. A common observation is that symptoms of prostatitis become more severe when the patient is under increased mental or physical stress. However, these observations have not been experimentally elucidated.

Setchell and Waites [19] and Glover [6] showed that stress induced by starvation and low temperature, respectively, results in decreased blood perfusion of the testis in rats. It has been reported that catecholamines reduce the blood flow in the testis and epididymis [3, 19]. Since stress is concomitant with an increased output of catecholamines [10] in blood, it appears to be logical to assume that the blood flow would be decreased in the testis, epididymis and prostate gland during stress. This assumption is justified by the reports of Selstam and Damber [18]. They have shown that the blood flow in the prostate is testosterone-dependent and that decreased concentration of testosterone in the blood results in decreased blood perfusion of the prostatic gland.

Perry [15] has shown that adrenaline suppresses the reproductive activity of English sparrows. He considered this to be due to the antagonizing effect of adrenaline on the pituitary gonadotropins [16]. It is thus uncertain whether the increased concentration of catecholamines in blood caused by stress has a direct effect on the vascular bed in the prostate gland or whether the reduction in blood flow is due to interference by the catecholamines with the production and/or effect of the pituitary gonadotropins.

In order to investigate whether blood perfusion of the prostate gland would decrease during stress stimulation, we employed the radioactive microsphere technique [4, 17] and examined male rats subjected to "short- and long-term" stress timuli.

In a parallel study, male rats were subjected to corresponding stress stimuli to those used in the present investigation [5]. In these rats it was found an increased concentration of catecholamines in arterial blood compared to the corresponding concentration measured in the controls, indicating that they had been subjected to stress stimuli. We have allowed ourselves to consider that these results indicate that also the animals in the present study have been subjected to stress stimuli.

The increased blood glucose concentrations measured after the laparatomies could be a stress effect [8, 9]. The decreased blood glucose concentrations measured in the rats subjected to "prolonged" stress stimuli are probably due to the starvation during the ninth day of the stress stimulation procedures just prior to the sampling (Table 1).

Blood flow in the kidneys was measured in all the tested animals. The agreement of blood flow between the paired kidneys indicates homogeneous mixing of the microspheres in the blood during measurements and consequently the accuracy of the obtained blood flow values.

The results show that the prostate gland of a rat subjected to experimental "short-term" stress stimuli reacts with a decrease of its blood flow – approximately 50 per cent under the experimental conditions of this study. The reduction was concomitant with corresponding reductions in the kidneys as well as in the testicles and seminal vesicles. The cardiac output decreased in contrast to the mean arterial blood-pressure which remained unchanged.

Decreased blood flows in the prostate gland, seminal vesicles and testicles were recorded in male rats subjected to standardized stress stimuli over a period of 10 days. The decreases were of the same magnitude as those recorded in the rats subjected to "short-term" stress stimuli procecures.

The mechanism underlying the reduction in blood perfusion of the prostatic gland during stress stimulation is not yet clarified. Stress stimuli are accompanied by a decreased testicular blood flow. Consequently it could be considered as probable that this would result in a reduced testosterone output affecting the prostatic gland and decreasing the prostatic blood flow [18]. However, the effects of a direct influence of catecholamines or nerve-mediated effects on the vascular bed in the prostatic gland are not ruled out as important factors in this context.

The genital organs of the rats which had been subjected to "prolonged" stress stimuli had decreased in wet weight. The prostate gland decreased more in wet weight than the corresponding relative decrease in total body weight, this in contrast to the testicles and seminal vesicles.

All recorded weights are given as wet weights and in figures relative to body weight of the corresponding animal. Precaution was taken to avoid drying of the organ specimens. For methodological reasons, relative weights instead of the common dry weights had to be used.

The atrophy of the prostatic gland, read as a loss of its relative wet weight is an indication of that the decrease in blood flow has been in force over a period to time. The loss of wet weight would not necessarily be related to a concomitant change of the total volume of the gland. It might for instance be due to a reduced amount of secretion in the gland. Whether the observed atrophy of the prostatic gland is accompanied by any histopathological changes has not been investigated in the present study.

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