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Gender Differences in the Effects of Streptozotocin-Induced Diabetes on Parasympathetic Vasodilatation in the Rat Submandibular Gland

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Abstract

Objectives—Submandibular vasodilatory responses are impaired in male streptozotocin-diabetic rats. However, the effects of diabetes on submandibular vascular reactivity in female rats have not been examined. The purpose of this study was to determine whether there are gender differences in the effects of diabetes on parasympathetic vasodilatation in the rat submandibular gland.

Methods—Diabetes was induced using streptozotocin, and vascular responses (calculated as the % increase in submandibular vascular conductance) to parasympathetic stimulation (1–10 Hz) were measured using laser-Doppler flowmetry. To estimate the relative contributions of nitric oxide (NO), prostacyclin (PGI₂) and endothelium-derived hyperpolarizing factor (EDHF), vascular conductance was measured before and after inhibition of cyclooxygenase (COX) and NO synthase (NOS).

Results—Frequency dependent increases in blood flow were observed in both male and female rats, but the contribution of EDHF was greater in females than in males. Further, PGI₂ appeared to play a role only in males. Vasodilatory responses were diminished in all diabetic animals, and when compared with their respective controls the degree of impairment was similar in males and females. However, in diabetic males inhibition of COX and NOS had little or no effect, whereas inhibition of NO, but not COX, resulted in a further significant decrease in vascular responses in diabetic females.

Conclusions—Parasympathetic vasodilatation in the rat submandibular gland is diminished equally in diabetic males and females. However, in males diabetes predominantly impairs PGI₂- and NO-dependent vasodilatation, whereas in females the contribution of EDHF-mediated pathways are affected and NO-dependent vasodilatation is preferentially maintained.

Keywords

Endothelium; Prostacyclin; Nitric Oxide; EDHF

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1. Introduction

Endothelial cells regulate vascular tone, in part, via the production of potent vasodilators such as nitric oxide (NO), prostacyclin (PGI₂) and endothelium-derived hyperpolarizing factor (EDHF). The deleterious effects of diabetes mellitus on vascular physiology are well documented and endothelial dysfunction represents a key event in the development of micro- and macrovascular complications in diabetes¹. Deterioration of the microcirculation begins early in the progression of the disease, and it underlies the development of the most serious complications associated with diabetes, e.g. cardiovascular disease, retinopathy, nephropathy and neuropathy². Amongst the major hallmarks of microangiopathy are functional changes in vascular tone and reactivity, and characteristic changes in blood flow occur in all affected vascular beds.

Gender differences in the relative contributions of endothelium-derived vasodilators to the local regulation of blood flow have been studied extensively^{3,4}, and there is general agreement that these differences are related to both genomic and non-genomic actions of sex hormones. Estrogen, progesterone and testosterone all have cardiovascular effects, but estrogen, in particular, is thought to favorably affect cardiovascular function³. The beneficial effects of estrogen are thought to be responsible for much of the lower risk for cardiovascular disease observed in 30–50 year old women compared with men of the same age. However, this gender advantage is lost following the onset of diabetes⁵. In addition, women are more likely to develop diabetic complications when compared with men, and they are at a greater disadvantage once complications do develop⁶.

Anderson & Garrett⁷ demonstrated that parasympathetic vasodilatation is impaired in the submandibular glands of male diabetic rats. Vasodilatation in response to direct parasympathetic nerve stimulation was reduced in 3 to 4 week streptozotocin diabetic rats through mechanisms involving both NO and PGI₂. More recently, gender differences in parasympathetic vasodilatation have also been documented in the rat submandibular gland⁸. Nitric oxide, PGI₂ and a presumptive EDHF all play a role in parasympathetic vasodilatation, but NO and an as yet uncharacterized EDHF appear to be the major endothelium-derived vasodilators in the rat submandibular gland. More importantly, EDHF was found to make a greater contribution in females than in males. However, the potential interaction between gender and diabetes on submandibular gland blood flow has not been investigated.

The purposes of this study, therefore, were to determine 1) whether gender influences the effects of diabetes on parasympathetic vasodilatation in the submandibular gland of the rat and 2) whether the relative contributions of NO, PGI₂ and EDHF to nerve-induced vasodilatory responses differ between diabetic males and females.

2. Materials and Methods

2.1 Animals

A total of 57 Sprague-Dawley rats (Simonsen Laboratories Inc., Gilroy, CA) were used in this study. Initial body weights (ages) were 220–240g (8–9 weeks) for males and 200–220 g (10–11 weeks) for females. Prior to induction of diabetes, all animals were allowed to acclimate for one week, and then divided into four groups: Male controls (n=17), male diabetics (n=10), female controls (n=18) and female diabetics (n=8). All animals were maintained on a 12h:12h, light:dark cycle and allowed free access to food and water. Housing conditions and experimental protocols were approved by the Animal Use and Care Committee of the University of the Pacific.

After an overnight fast, diabetes was induced by the intraperitoneal injection of streptozotocin (58 mg kg^{-1} , Sigma Chemical Co., St. Louis MO). Blood glucose levels were measured using a standard glucose test meter (OneTouch®, Johnson & Johnson) and only animals having non-fasting glucose levels $> 250 \text{ mg/dl}$ were considered diabetic for the purposes of this study.

2.2 Parasympathetic Nerve Stimulation

Four to six weeks after the induction of diabetes, submandibular blood flow responses to parasympathetic stimulation were measured using laser-Doppler flowmetry. Previous studies have shown that over this range of disease duration the effects of diabetes on salivary gland physiology relatively are stable⁹. Anesthesia was induced with sodium pentobarbital (35 mg kg^{-1} , i.p.) followed by chloralose (80 mg kg^{-1} , i.v.). For parasympathetic nerve stimulation, the chorda-lingual nerve was exposed and carefully reflected onto the submandibular duct. Both the duct and the nerve were then placed on a bipolar electrode and parasympathetic impulses were delivered at consecutively higher frequencies of 1, 2, 5 and 10 Hz (5–6 V, 2 ms duration) for 90 seconds at each stimulation frequency. Generally, there was a 3 to 5 minute interval between each successively higher stimulation frequency to allow blood flow to return to base-line and stabilize.

The relative contributions of PGI_2 and NO to parasympathetic vascular responses were estimated by sequentially inhibiting cyclooxygenase (COX) and NO synthase (NOS). Cyclooxygenase activity was inhibited by the intraperitoneal administration of indomethacin (5 mg kg^{-1}), and NO production was then blocked by the continuous intravenous infusion of NG -nitro-L-arginine methyl ester (L-NAME, $10\text{--}15 \text{ mg kg}^{-1} \text{ minute}^{-1}$). The remaining non- PGI_2 , non-NO vasodilatation was attributed to EDHF. After the initial introduction of each inhibitor there was a 20–30 minute wait before beginning the next series of stimulations. The reciprocal order for the inhibitors was not performed, because previous work demonstrated that the order made no difference in females and in males the role of PGI_2 was observed only when cyclooxygenase was inhibited before blocking NOS⁹.

To ascertain whether neurotransmitter depletion might affect vascular responses, a final set experiments was carried out to measure the effects of multiple stimulation protocols (1, 2, 5 and 10 Hz). Male ($n=2$) and female ($n=2$) control rats were anesthetized and three stimulation sequences were carried out, as described above, with the exception that no inhibitors of NOS or COX were administered.

Systemic mean arterial blood pressure (MABP) was monitored continuously via a blood pressure transducer placed in the femoral artery. The trachea was cannulated to ensure a clear airway, but the animal was not artificially ventilated. Body temperature was maintained between 37°C and 38°C using a heated surgical table.

At the end of each experiment the animal was euthanized by an intracardiac administration of sodium pentobarbital (50 mg kg^{-1}). The left, unstimulated glands were then immediately removed, blotted on filter paper and weighed. In females, the uterus was also removed and weighed.

2.3 Laser-Doppler Flowmetry

The posterior, lateral quadrant of each right submandibular gland was exposed by creating a small window through the skin and overlying subcutaneous tissue. A laser-Doppler stainless steel probe (MP3a, Moor Instruments, London, UK) was placed at right angles to the gland without applying pressure to the tissue. Previous experience with laser-Doppler flowmetry suggested that placement of the probe too close to the hilum of the gland resulted in very high resting perfusion values (arbitrary flux units >300), probably due to the probe being placed

over a major branch of the submandibular artery (unpublished observations). Moving the probe away from this region led to an immediate reduction in the perfusion readings to between 100 and 200 flux units. For each animal the probe was positioned to give an initial baseline reading of between 130 and 160 flux units, and once positioned the probe was positioned it was not moved again for the duration of the experiment. At the end of each stimulation period, blood flow was allowed to return to baseline before the next stimulus was applied. After the administration of each inhibitor, a twenty to thirty minute waiting period was observed before the series of parasympathetic stimulations was repeated.

Laser-Doppler flowmetry measures blood flow in arbitrary perfusion units (laser-Doppler flux, LDF), but for statistical purposes submandibular vascular conductance (SVC) was calculated as LDF divided by MABP. The vasodilatory responses to parasympathetic stimulation were then expressed as the integral calculated as the sum of the data points times the duration of stimulation ($SVC_{stimulated}$) minus the integral calculated as the sum of the data points less the first value times the duration of the stimulation (SVC_{basal}). All data were captured, stored and analyzed using PowerLab© acquisition software (ADInstruments, Colorado Springs, CO).

2.4 Statistical Analysis

All data were expressed as mean $\pm$ standard error of the mean, and then analyzed using statistical software (SigmaStatR). To test for interactions between gender and diabetes the data were analyzed using a two-way analysis of variance (ANOVA) with the alpha level set at 0.05. Within each group (e.g. male controls) data were analyzed for statistical significance using a one-way repeated ANOVA with the alpha level set at 0.05. As appropriate, differences between individual means (control vs. indomethacin or indomethacin + L-NAME) were then tested using the Holm-Sidak test for multiple comparisons. To test for effects of gender on indomethacin and L-NAME inhibition of vascular responses in diabetic animals, data were analyzed using a mixed two-way analysis of variance (MANOVA).

3. Results

3.1 Blood Glucose, Body and Gland Weight, Uterine Weight

Final body weights, gland weights and in females uterine weights are given in Tables 1 and 2. The induction of diabetes resulted in a significant increase in serum glucose ($p < 0.001$) and, as judged by the level of hyperglycemia, there was no difference between male and female diabetic rats with respect to the severity of diabetes induced. In addition, four to six weeks after the onset of hyperglycemia, both body and submandibular gland weights were lower in diabetic animals than in age-matched, non-diabetic controls. Finally, in females the induction of diabetes resulted in a significant decrease in both uterine weight and uterus to body weight ratio ($p < 0.001$).

3.2 MABP, Basal Submandibular Vascular Conductance

Under pentobarbital anesthesia MABP was similar in all experimental groups; 133 ± 24 mm Hg (male non-diabetic), 114 ± 9 mm Hg (male diabetic), 116 ± 6 mm Hg (female non-diabetic) and 106 ± 8 mm Hg (female diabetic). Indomethacin had no apparent effect on MABP, but the administration of L-NAME resulted in a rapid and sustained increase in MABP (+39% and +40% in control and diabetic males, and +22% and +25% in control and diabetic females). As a consequence of this pressor response, glandular perfusion calculated as SVC_{basal} (LDF/MABP) was significantly reduced following the administration of L-NAME (−32% and −33% in control and diabetic males, and −27% and −34% in control and diabetic females). However, for any given animal SVC_{basal} remained stable over repeated parasympathetic stimulations under each condition tested, and after each stimulation period SVC_{basal} returned to the values recorded prior to stimulation.

3.3 Effects of Diabetes and Gender on Parasympathetic Vasodilatation

In the absence of inhibitors of NOS or COX, parasympathetic stimulation elicited frequency-dependent increases in SVC_{stim} (Figures 1 and 2). When expressed as a percent increase above SVC_{basal} (mean $\pm$ S.E.M.), there were no differences with respect to the increase in SVC_{stim} between male ($+32 \pm 4\%$ at 1 Hz, $+82 \pm 9\%$ at 2 Hz, $+206 \pm 18\%$ at 5 Hz and $+282 \pm 18\%$ at 10 Hz) and female non-diabetic, control rats ($+36 \pm 5\%$ at 1 Hz, $+75 \pm 10\%$ at 2 Hz, $+201 \pm 13\%$ at 5 Hz and $+289 \pm 22\%$ at 10 Hz).

Frequency-dependent increases in SVC_{stim} were also observed in male and female diabetic animals. However, in diabetic rats the percent increases in vascular conductance were significantly lower at all frequencies when compared with those observed in age-matched, controls ($p < 0.05$). Further, the effects of diabetes on SVC_{stim} were not statistically different between males and females. The percent reductions in SVC_{stim} in diabetic males and females compared with non-diabetic controls were -38% and -42% at 1 Hz, -46% and -47% at 2 Hz, -39% and -36% at 5 Hz and -38% and -29% at 10 Hz, respectively.

3.4 Effects of Diabetes and Gender on the Relative Contributions of NO, PGI_2 and EDHF to Parasympathetic Vasodilatation

The effects of indomethacin alone or in combination with L-NAME on parasympathetic vasodilatation in control and diabetic animals are shown in Figure 3. In male control rats the addition of indomethacin (Fig. 3A) significantly reduced parasympathetic vasodilatation at 1 Hz (-50%), 2 Hz (-37%) and 5 Hz (-24%), $p < 0.05$, but had no apparent effect at 10 Hz (9%). In contrast, indomethacin had no effect on parasympathetic vasodilatation in the submandibular glands of control female rats. Following the addition of L-NAME there was a further reduction in parasympathetic vasodilatation at all stimulation frequencies in males. The total decreases in SVC_{stim} in the presence of both indomethacin and L-NAME were -78% at 1 Hz, -63% at 2 Hz, -41% at 5 Hz and -30% at 10 Hz. In females (Fig 3B), L-NAME also further reduced SVC_{stim} , but particularly at 5 Hz and 10 Hz the effects of blocking NO synthesis on SVC_{stim} were significantly less than in males (-62% at 1 Hz, -50% at 2 Hz, -19% at 5 Hz and only -9% at 10 Hz).

The effects of indomethacin alone or in combination with L-NAME on parasympathetic vasodilatation in diabetic animals are shown in Figures 3C and 3D. In both diabetic males and females, the administration of indomethacin had no significant effect on SVC_{stim} at any stimulation frequency. In contrast, the effects of adding L-NAME appeared to be gender-dependent, and MANOVA confirmed that there was a significant interaction between gender and diabetes. In diabetic male rats, L-NAME led to a significant reduction in SVC_{stim} at 1 Hz only (total decrease was -53% , $p < 0.05$), whereas the addition of L-NAME in diabetic female rats resulted in significant decreases in SVC_{stim} of -52% at 1 Hz, -60% at 2 Hz, -46% at 5 Hz and -35% at 10 Hz ($p < 0.05$).

Finally, to rule out the possibility that neurotransmitter depletion might have affected the results, blood flow responses were measured during three successive repetitions of the stimulation protocol in two male and two female control rats (Figure 4). No statistical differences were observed between the first series of stimulations and the third series at any of the frequencies tested.

4. Discussion

Salivary glands are supplied with an extremely dense microvascular network, and both sympathetic and parasympathetic nerves influence glandular blood flow. Parasympathetic nerve stimulation evokes a copious salivary flow that is accompanied by an immediate increase

in glandular perfusion, which is thought to maintain tissue hydration during active secretion⁹. In the submandibular gland of the rat, salivary flow is due mainly to the activation of muscarinic receptors on the acinar cells by acetylcholine. However, substance P also plays a role in submandibular secretion⁸. In contrast, increases in submandibular perfusion are attributable largely to the synthesis and release of endothelium-derived vasodilators in response to acetylcholine, substance P and vasoactive intestinal peptide (VIP)^{10,11}. Further, both NO-dependent and NO-independent mechanisms play a role in endothelial regulation of vascular tone in the submandibular gland⁹. Previous studies demonstrate that submandibular vascular responses to parasympathetic stimulation are gender-dependent and that both estrogen and progesterone influence the relative contributions of specific endothelium-derived vasodilators^{8,12}. Nitric oxide, PGI₂ and EDHF all contribute to the parasympathetic vascular responses in the rat submandibular gland, but NO and EDHF are the predominant endothelium-derived vasodilators irrespective of gender⁸. Nonetheless, gender differences were observed when both PGI₂ and NO synthesis are blocked. Under those conditions the remaining vasodilatory responses, presumed to be EDHF-dependent, were significantly lower in males than in females. Furthermore, in females the loss of estrogen (ovariectomy) diminishes submandibular vascular responses to parasympathetic stimulation, and all three pathways are affected¹².

Our present observations extend the findings from previous studies on gender and submandibular vascular reactivity by demonstrating that the effects of diabetes on parasympathetic vasodilatation in the rat submandibular gland differ between males and females. Although the negative effects of diabetes on overall vasodilatory responses to parasympathetic stimulation appear to be similar in males and females, this masked a significant gender difference in the relative contributions of NO, PGI₂ and EDHF. Specifically, impairment of parasympathetic vasodilatory responses in diabetic males appeared to be due mainly to the loss of NO and PGI₂, whereas in females the role of NO was maintained, or even slightly enhanced and that of EDHF was reduced.

Numerous studies have demonstrated that the relative contribution of each of the endothelium-derived mediators to vasodilatation depends on the species, the vascular bed under study (e.g., mesenteric vs. cerebral vessels) and vessel size¹³. Nitric oxide generally predominates in large conductance vessels, whereas EDHF becomes increasingly important as vessel size decreases. Thus, it is not surprising that EDHF appears to play a prominent role in submandibular vasodilatation.

As observed in an earlier study⁸, a presumptive EDHF played a larger role in non-diabetic female submandibular glands than in glands from non-diabetic male rats. Gender differences in vascular reactivity have been widely studied^{3,14,15}, and our observations are in agreement with a growing body of evidence suggesting that in resistance vessels the predominant mediators contributing to endothelium-dependent vasodilatation in males are NO and PGI₂, whereas in females EDHF is more important^{16–18}. These gender differences with respect to the relative contributions of endothelium-derived vasodilators are due in large measure to the influence of sex hormones on endothelial cell function. Estrogen, progesterone and testosterone all have cardiovascular effects, but estrogen in particular is thought to provide numerous cardiovascular benefits through its effects on the synthesis and release of both NO and EDHF^{3,19,20}.

Although the vasodilatory responses remaining after inhibition of both COX and NOS were attributed to EDHF, there is no direct evidence in the submandibular gland to definitively conclude that EDHF alone was responsible. Further, EDHF is unlikely to be a single species, and has been variously proposed to be H₂O₂, cAMP, one of several products of the epoxigenase pathway, K⁺ ions, the spread of current through myoepithelial gap junctions and C-type

naturetic peptide²¹. Because of the elusive nature of EDHF, selective antagonists of EDHF have yet to be identified, and as a result EDHF activity is regularly defined *in vivo* as the vasodilatory response that remains after combined inhibition of NO and PGI₂ synthesis. However, the attribution of non-NO, non-PGI₂-dependent vasodilatation to the action(s) of EDHF may be problematic, if endothelium-independent mechanisms are involved to any significant degree in the vasodilatory responses to parasympathetic stimulation.

Two potential endothelium-independent pathways exist: the release of NO from parasympathetic nerves and a direct vasorelaxant effect of VIP on vascular smooth muscle. Immunocytochemical localization indicates that neuronal NOS (nNOS) is present in parasympathetic nerves surrounding acinar cells, ducts and blood vessels in rat salivary glands^{22–24}. Although there is little direct evidence to support a role for neuronally-derived NO in salivary vascular responses, inhibition of nNOS using N^ω propyl-L-arginine (N-PLA) significantly reduced vasodilatory responses to parasympathetic nerve stimulation in the rat submandibular gland⁸. At very low stimulation frequencies (1 Hz or less), nNOS appeared to be responsible for all of the NO-mediated increases in submandibular blood flow. However, as stimulation frequency was increased, the contribution of endothelial NOS (eNOS) appeared to rapidly overwhelm that of nNOS. These data, while not conclusive, support the hypothesis that neuronally-derived NO contributes to NO-dependent vasodilatation in the rat submandibular gland.

In contrast, VIP is thought to influence blood flow via two distinct mechanisms. The first is endothelium-dependent and involves the release NO, whereas the second pathway is endothelium-independent and is mediated by a direct effect on vascular smooth muscle (through the elevation of intracellular cAMP)²⁵. Although VIP is present in parasympathetic nerves supplying the rat submandibular gland, recent evidence suggests that an endothelium-independent effect of VIP glandular blood may be minimal⁷. In the presence of both atropine and L-NAME, parasympathetic vasodilatation was significantly reduced by greater than 60%. The remaining vasodilatory response was virtually abolished by the addition of a specific competitive tachykinin receptor antagonist (spantide I) that blocks the actions of substance P. Substance P regulates vasodilatation and plasma extravasation via binding to endothelial NK1 receptors²⁶. These data do not rule out a role for VIP in parasympathetic vasodilatation in the rat submandibular gland, because NO synthesis was blocked prior to the addition of spantide. However, they do strongly suggest that, apart from a minor role for neuronally-derived NO, there is no significant endothelium-independent vasodilatory response to parasympathetic stimulation in the rat submandibular gland.

Serum levels of both progesterone and estrogen are reduced in streptozotocin-induced diabetic female rats²⁷, and a decrease in estrogen receptor alpha levels in female diabetic rats has also been reported²⁸. Interestingly, several studies suggest that in mesenteric arteries NO-mediated relaxation is relatively unaffected or even decreased by estrogens, whereas as the contribution of EDHF is increased^{29–31}. Thus, one possible explanation for the gender differences observed in this study is that a decrease in circulating levels of estrogen or a reduction in estrogen receptors in diabetic female rats was responsible for the preferential loss of EDHF-mediated vasorelaxation. Although we did not measure plasma levels of estrogen, the reduction in uterine weight in our female diabetic animals supports the hypothesis that a disruption in sex hormone metabolism underlies the observed gender differences in rat submandibular vasodilatation following the induction of diabetes.

It must be noted that the present study was designed to investigate the effects of gender, rather than circulating estrogen levels *per se*, nor was the phase of the estrous cycle determined on the day of each experiment involving female animals. Based on our previous observations in 16 week old adult female rats¹⁹, and a report by Moien-Afshari and coworkers³², we can

conclude that estrogen levels in females are stable over time (3 to 21 months). Our female rats were 16–18 weeks of age at the time of blood flow measurements, which is approximately 8–12 weeks after adult estrogen levels are attained in the rat. Therefore, while some of the variability in vascular responses in female rats might have been due to changes in circulating levels of estrogen in normally cycling animals, this variability would not appear to significantly impact our conclusions.

The potential role of testosterone in submandibular dysfunction in male diabetic animals, however, is somewhat more difficult to speculate upon. Testosterone does have beneficial vascular effects, including promoting the release of endothelium-derived relaxing factors (particularly NO) and hyperpolarization of vascular smooth muscle³. Further, streptozotocin diabetes has been associated with testicular dysfunction and a reduction in serum testosterone levels in male diabetic animals³³, and low testosterone levels have also been associated with endothelial dysfunction and an increased risk for cardiovascular disease in men^{34,35}. Thus, a decrease in circulating testosterone levels might have led to the observed loss of NO-mediated submandibular vasodilatation in the male diabetic rats. However, in contradiction to the clinical findings cited above there is evidence to suggest that castration may actually improve vascular function in male diabetic rats³⁶.

With respect to the role of EDHF, there are no reports of studies that examined the effects of gender and diabetes, although impaired EDHF-mediated mechanisms have been associated with endothelial dysfunction in experimental models of both type 1 and type 2^{37–42}. Conversely, an augmented EDHF response was shown to compensate for the loss of NO-mediated vasorelaxation in femoral and mesenteric arteries in streptozotocin-induced diabetic rats⁴³. All of these studies were conducted using only in male animals, and most utilized an in vitro mesenteric artery preparation. Consequently, it remains to be seen whether gender-specific effects of diabetes on EDHF-mediated vascular responses in the submandibular gland are specific to the experimental protocol (*in vivo* nerve stimulation) and vascular bed, or whether they can be more generally applied to the problem of endothelial dysfunction in diabetes.

Lastly, in addition to its effects on NO synthesis L-NAME has been reported to have affinity for muscarinic receptors and to act as a muscarinic receptor antagonist *in vitro*⁴⁴. This has resulted in some caution being expressed about the interpretation of the observed effects of L-NAME on blood flow *in vivo*⁴⁵, including those reported in a recent study looking at impaired submandibular vascular responses to parasympathetic nerve stimulation in nonobese diabetic mice⁴⁶. However, other data suggest that although L-NAME may have affinity for muscarinic receptors *in vitro*, it does not have significant anti-muscarinic activity *in vivo*^{47–49}. Further, we have been unable to demonstrate any effect of L-NAME on submandibular salivary flow rate (unpublished data), which suggests that *in vivo* L-NAME has little effect on muscarinic receptors in the rat submandibular gland.

In conclusion, we have demonstrated that in the rat submandibular gland short-term streptozotocin-induced diabetes (4–6 weeks duration) results in a diminished vasodilatory response to parasympathetic stimulation, and that the overall degree of impairment is similar in both male and female rats. However, when the relative contributions of NO, PGI₂ and a putative EDHF were compared, the data revealed significant gender-based differences. In males, the reduction in vasodilatory responses appeared to be due to a loss of NO- and PGI₂-dependent vasorelaxation, whereas in females it was the EDHF-dependent response that was affected by the induction of diabetes. Thus, our observations support the hypothesis that the specific effects of diabetes on vascular responsiveness are gender-dependent. Particularly in females, the shift from away from EDHF as the major vasodilatory factor in peripheral vessels

toward a greater reliance on NO may be directly related to the increased risk for cardiovascular complications in female patients with diabetes mellitus compared with their male counterparts.

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Figure 1. Representative Recordings of Vascular Conductance in Male and Female, Control and Diabetic Rat Submandibular Glands during Parasympathetic Stimulation

Changes in vascular conductance were measured at 1, 2, 5 and 10 Hz stimulation (5–6 v and 2 ms duration) of the chorda-lingual nerve. Each stimulation period was for 90 seconds.

Figure 2. Effects of Gender and Diabetes on Parasympathetic Vasodilatation in the Rat Submandibular Gland

Changes in perfusions were expressed as the per cent increase in vascular conductance above basal conductance. Each bar represents the mean $\pm$ s.e.m. for male, non-diabetic (n=17), male, diabetic (n=10), female, non-diabetic (n=18), female, diabetic (n=8). * $p < 0.05$ male, non-diabetic vs male, diabetic; ** $p < 0.05$ female, non-diabetic vs female, diabetic.

Figure 3. Effects of Inhibiting PGI₂ and NO Synthesis on % Increases in Submandibular Vascular Conductance

Perfusion was measured in the presence of indomethacin (5 mg kg⁻¹, i.p.) followed by continuously infused L-NAME (10–14 mg min⁻¹ kg⁻¹, i.v.). Each point represents the mean ± s.e.m. percent increase in perfusion: Panel A, Male, non-diabetic control rats (n=17); Panel B, Female, non-diabetic control rats (n=18); Panel C, Male diabetic rats (n=10); Panel D: Female diabetic rats (n=8). Repeated measures ANOVA: * Control vs Indomethacin + L-NAME (p<0.05); ** Control vs Indomethacin and control vs Indomethacin + L-NAME (p<0.05); ***All differences significant (p<0.05).

Figure 4. Effect of Multiple Parasympathetic Stimulations on Vascular Conductance in Male and Female Rats

Changes in vascular conductance were measured over three separate stimulation sequences (1, 2, 5 and 10 Hz, 5–6 v and 2 ms duration) of the chorda-lingual nerve. Each stimulation period was for 90 seconds, and there was a 20 minute waiting period between each sequence. Each bar represents the average blood flow responses observed in two animals.

Table 1

Serum Glucose and Body and Gland Weights in Male Rats

	Serum Glucose (mmol/l)	Body Weight (g)	Gland Weight (mg)
Control (n=17)	133 ± 14	352 ± 20	288 ± 26
Diabetic (n=10)	445 ± 76*	245 ± 27*	198 ± 24*

Mean ± S.D,

*
p<0.001, Diabetes vs Control

Table 2

Serum Glucose, and Body and Gland and Uterine Weights in Female Rats

	Serum Glucose (mmol/l)	Body Weight (g)	Gland Weight (mg)	Uterus (mg)	Uterus/Body (mg/g)
Control (n=18)	126 ± 16	260 ± 9	208 ± 5	531 ± 88	2.05 ± 0.38
Diabetes (n=8)	504 ± 82 *	216 ± 5 *	132 ± 10 *	299 ± 33 *	1.41 ± 0.12 *

Mean ± S.D.,

* p<0.001, Diabetes vs Control