Retinal Circulation Responses to Systemic Autonomic Nerve Stimulation.

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Summary

The retinal vessel calibre responses to controlled stimulation of the autonomic nervous system were studied in 10 healthy subjects, using sustained isometric muscle contraction as stimulus. Each subject was studied twice using different mydriatic agents, (1) g.tropicamide 1% a parasympatholytic agent and (2) g. phenylephrine 10% a sympathetic agonist. In the tropicamide study, there was a mean arteriolar constriction of 8.1% (SEM 1.67, $p < 0.001$) and venule constriction of 3.7% (SEM 0.85, $p < 0.001$) with a mean rise in diastolic blood pressure of 27.4 mmHg (SEM 2.95, range: 13-45 mmHg). When g.phenylephrine 10% was used, there was a mean arteriolar constriction of 8.6% (SEM 1.68, $p < 0.001$) and venule constriction of 4.8% (SEM 1.22, $p < 0.001$) with a mean rise in diastolic blood pressure of 29.2 mmHg (SEM 2.56, range: 17-44 mmHg). There was no significant difference in retinal vessel calibre in the recovery phase compared to baseline phase ($p > 0.05$) or between the two mydriatic agents on vessel responses ($p > 0.05$). There was no correlation between the rise in diastolic blood pressure and the degree of retinal vessel constriction, during handgrip contraction in either study. This study has demonstrated a significant association between retinal vessel calibre and systemic autonomic nerve stimulation. The possible mechanisms for the retinal vessel constriction observed in this study are discussed.

The retinal circulation is autoregulated¹ and therefore has the capability to maintain constant blood flow despite changes in perfusion pressure whether induced by changes in intraocular pressure or arterial blood pressure.²⁻⁴ The mechanisms which control this autoregulation are poorly understood.⁵

The aim of the present study was to assess the responses of the retinal circulation to controlled stimulation of the autonomic nervous system in normal subjects. A number of well established tests based on cardiovascular reflexes allow accurate assessment of autonomic nerve function.⁶⁻¹⁰ Sustained

handgrip provides an isometric muscle contraction which provokes a sympathetic response with reflex rise in diastolic blood pressure.^{9,11} This pressor response is presumed to be initiated by stimuli from the exercising muscle.¹² The rise in diastolic blood pressure is partly due to peripheral vasoconstriction secondary to activation of the alpha adrenergic receptors of the peripheral autonomic nervous system¹³ and partly to a heart-rate dependent increase in cardiac output.¹⁴ A diastolic blood pressure rise of ≤ 10 mmHg in response to sustained handgrip is abnormal, a rise of ≥ 16 mmHg is

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normal and the response is borderline if between 10 and 16 mmHg.¹⁵

Patients and Methods

After informed consent had been obtained, 10 healthy subjects were included in the study: their mean age was 30 years (range 23–35 years) and 6 were male. Each subject was studied seated at a fundus camera on two occasions: one pupil was dilated (whichever was preferred by the subject) with g. tropicamide 1% (a parasympatholytic agent) for the first study and g. phenylephrine 10% (a sympathetic agonist) for the second. Retinal vascular responses to sympathetic nerve stimulation were measured separately using pupillary dilating agents with different autonomic effects, to determine the effect of topical autonomic agonists on retinal vessel responses. All photographs were taken by the same observer using a wide angle Canon fundus camera (CF 60-S) which was triggered during the diastolic phase of the pulse cycle, using a finger-tip pulse detector coupled to an adjustable time delay. Five photographs were taken in quick succession at approximately one second intervals at each phase.

Baseline photographs were taken followed by 3 baseline measurements of blood pressure recorded from the non-exercising arm (Fig. 1) using an automatic sphygmomanometer (COPAL-UA 231). The subject was then instructed to perform 3 maximum handgrip contractions, using the dominant arm, to establish the maximum voluntary contraction (MVC). The duration of each maximal contraction lasted less than 2 seconds. A laboratory built strain gauge

handgrip dynamometer with continuous chart recording was used; it had an adjustable audible alarm set at 33% of each subject's MVC. The subject was then instructed to perform a sustained handgrip at 33% MVC for 2.5 minutes. During this exercise period 3 phases of fundus photographs were taken at 1, 2 and 2.5 minutes; three blood pressure measurements were recorded at 0.5, 1.5 and 2.5 minutes (Fig. 1). On recovery one phase of fundus photographs and a final blood pressure reading were taken at five minutes after release of sustained handgrip contraction. All photographs were recorded on Ilford FP4 film with a red free filter and developed in PQ Universal (1 + 19) for 12 minutes at 20°C with sensitometric control.¹⁶

Analysis:

The films were analysed using the Quantimet 800 Image Analyser (Cambridge Instruments). This technique has been developed in the Department of Ophthalmology, Royal College of Surgeons of England.¹⁶ Seventeen suitable vessel sites were measured in each subject comprising similar numbers of arterioles and venules nominally within the calibre range: 60–170 μm (calculated on the basis of the Gullstrand Schematic Eye). A typical fundus photograph illustrating sites of measurement in one subject is shown in Figure 2. All calibre measurements of these vessel sites were stored on floppy disc for later retrieval and statistical analysis which included calculation of means and standard error of the means (SEM) and one way

Fig. 1. Protocol of handgrip study.

Fig. 2. Typical fundus photograph with measurement sites.

analysis of variance (A.N.O.V) within replications and between the phases for each site. Percentage change in mean retinal vessel calibre between phase 1 (pre-test) and phase 4 (following 2.5 minutes of sustained handgrip) for each subject was calculated; significance was assessed by the student t for paired data, 2 tailed test.

Results

The individual results of variation in retinal vessel calibre during sustained isometric muscle contraction are shown in Tables I and II. Group mean values are as follows:

(i) Pupil dilation with g.tropicamide 1%

In response to sustained handgrip, arteriolar calibre (mean $\pm$ SEM) decreased by 8.1%

± 1.67 ($p < 0.001$) and venule calibre decreased by $3.7\% \pm 0.85$ ($p < 0.01$); diastolic blood pressure increased by 27.4 ± 2.95 mmHg (range: 13–45 mmHg).

(ii) Pupil dilation with g.phenylephrine 10%

In response to sustained handgrip, arteriolar calibre decreased by $8.6\% \pm 1.68$ ($p < 0.001$) and venule calibre decreased by $4.5\% \pm 1.84$ ($p < 0.05$); diastolic blood pressure increased by 29.2 ± 2.56 mmHg (range: 17–44 mmHg).

There were no significant differences in retinal vessel calibre responses to sustained handgrip or diastolic blood pressure between the dilating agents ($p > 0.05$). However inadequate mydriasis often resulted when g.phenylephrine 10% was used in subjects with dark irides. There were no significant

Table I Variation in retinal vessel calibre during sustained isometric muscle contraction in 10 normal subjects, following pupil dilation with g.tropicamide 1% (Mean $\pm$ SEM).

Subject Number	Variation in arterial calibre %	sig.	Variation in venule calibre %	sig	Rise in dias BP %, mmHg
1	-8.10 ± 1.63	$p < 0.001$	-0.50 ± 2.39	ns	28 23
2	-4.38 ± 2.85	ns	-7.14 ± 3.50	$p < 0.05$	28 21
3	-14.42 ± 2.66	$p < 0.01$	-1.50 ± 2.76	ns	17 13
4	-9.10 ± 4.45	$p < 0.01$	-7.75 ± 1.74	$p < 0.01$	40 27
5	-11.70 ± 1.04	$p < 0.001$	-4.13 ± 1.02	$p < 0.01$	36 27
6	-13.44 ± 2.38	$p < 0.001$	-4.22 ± 1.40	$p < 0.05$	42 33
7	-6.09 ± 1.35	$p < 0.05$	0 ± 2.45	ns	35 23
8	-5.44 ± 0.87	$p < 0.001$	-2.00 ± 1.13	ns	64 45
9	$+3.25 \pm 5.28$	ns	-3.75 ± 5.71	ns	46 39
10	-11.75 ± 3.04	$p < 0.01$	-5.77 ± 2.24	$p < 0.05$	32 23

Table II Variation in retinal vessel calibre during sustained isometric muscle contraction in 10 normal subjects, following pupil dilation with g.phenylephrine 10%. (Mean $\pm$ SEM)

Subject Number	Variation in arterial calibre %	sig.	Variation in Venule calibre %	sig.	Rise in Dias BP %, mmHg
1	-5.60 ± 2.83	$p < 0.05$	-0.28 ± 1.72	ns	31 26
2	-6.25 ± 4.85	ns	-6.14 ± 2.48	$p < 0.05$	35 26
3	-14.11 ± 8.06	ns	-17.00 ± 9.65	ns	51 37
4	-3.10 ± 3.26	ns	-5.98 ± 1.48	$p < 0.01$	43 30
5	-15.25 ± 1.33	$p < 0.001$	-5.44 ± 3.00	ns	62 44
6	-14.00 ± 4.54	ns	-1.33 ± 2.51	ns	34 24
7	-7.63 ± 1.89	$p < 0.01$	$+0.85 \pm 1.74$	ns	25 17
8	$+0.75 \pm 4.28$	ns	$+4.00 \pm 0.97$	$p < 0.01$	41 32
9	-7.71 ± 2.19	$p < 0.05$	-7.40 ± 4.66	ns	44 35
10	-12.77 ± 2.33	$p < 0.001$	-6.57 ± 2.64	$p < 0.05$	26 21

changes in either retinal vessel calibre or blood pressure in the recovery phase compared to the control phase ($p>0.05$). The reduction in venule calibre during these studies was less than the arterioles but nevertheless statistically significant. There was no correlation between the rise in diastolic blood pressure and the degree of retinal vessel constriction in either the tropicamide study ($r=+0.51$, $p>0.10$) or the phenylephrine study ($r=-0.187$, $p>0.50$).

Individual vessel calibre changes at 18 sites (Fig. 2), in a typical subject are shown in Table III. This demonstrates the variability of the individual vessel responses at different sites during sustained isometric muscle contraction. In this subject there was an overall mean arteriolar constriction of 11.7% (SEM 1.04, $p<0.001$) and venule constriction of

4.1% (SEM 1.02, $p<0.01$) with a mean rise in diastolic blood pressure of 27 mmHg.

In the tropicamide 1% study a total number of 90 arteriolar sites were measured of which 58 showed significant change ($p<0.05$, one way A.N.O.V) and 81 venule sites (46 significant, $p<0.05$). In the phenylephrine 10% study a total of 81 arteriolar sites (22 significant, $p<0.05$) and 69 venule sites (19 significant $p<0.05$) were measured.

Figure 3 shows the percent change in mean vessel calibre and diastolic blood pressure for all subjects through phases 2-5 (sustained handgrip contraction), compared to the mean baseline values in phase 1.

Discussion

The present study has shown consistent responses of the retinal vasculature to controlled sympathetic nerve stimulation, resulting from a well established test, isometric muscle contraction produced by sustained handgrip. These responses are consistent

Table III Individual retinal vessel calibre changes during sustained isometric muscle contraction in a single subject.

Subject No. 5 Age 29 G. Tropicamide 1%
Rise in diastolic BP - +27mmHg or +36%

		Percent change in vessel calibre (phase 4 compared with phase 1)
Arterial sites:		
No	1	- 12
	2 *	- 13
	3 *	- 11
	4	- 16
	5	- 6
	6 *	- 14
	7	- 6
	8 *	- 14
	9 *	- 12
	10 *	- 13
Venule sites:		
No	11	- 5
	12	- 5
	13	- 2
	14	- 5
	15	+ 1
	16	- 9
	17 *	- 5
	18	- 3

* significant sites A.N.O.V. ($p<0.05$)

Fig. 3. Percentage change in vessel calibre and diastolic blood pressure (mean $\pm$ SEM) in phases 2-5, compared with phase 1.

with autoregulation, in the face of the rising diastolic blood pressure which accompanies sustained handgrip (Fig. 3), since reduction in retinal arteriolar calibre implies an increase in peripheral resistance, which theoretically varies inversely as the fourth power of the vessel radius, according to Poiseuille's Law. The relationship between brachial blood pressure and ophthalmodynamometric retinal artery pressure in population studies is well known, and the consistent relationship when blood pressure was raised by isometric exercise was demonstrated by Robinson et al.²

There is extensive evidence for autoregulation in the retinal circulation^{3,4,17,18} including responses to acute rises in blood pressure produced by systemic infusions of angiotensin and noradrenaline,^{19,20} though the latter experiments have shown varying results. Retinal vessel constriction in the present study occurred within 30 seconds of commencing the sympathetic stimulation and coincided with the rise in diastolic blood pressure. With sustained stimulation during the period of handgrip both retinal vessel response and diastolic blood pressure elevation increased (Fig. 3); this would suggest a temporal relationship between autonomic nerve stimulation and retinal vessel response. Vascular smooth muscle tone may be influenced in three ways: neurogenic, myogenic or humoral. As yet there is no evidence of autonomic innervation of the retinal vessels, forward of the lamina cribrosa.²¹⁻²³ The retinal arterioles near the optic disc have 5 to 7 layers of smooth muscle.²² Russell¹⁸ reported that a 16% increase in retinal arteriolar calibre occurred, immediately following reduction of retinal perfusion pressure, produced by compression of the globe with a spring dynamometer: similar results were obtained both in patients with Horner's Syndrome and normal subjects. He argued that these observations pointed to the improbability of involvement of the sympathetic nervous system; and suggested a myogenic response due to altered transmural pressure (Bayliss effect,²⁴). There is however some uncertainty about the status of these patients since the degree and level of denervation was not defined.

In the sympathectomised eyes of cats Kassel et al.²⁵ have shown that acute elevation of the blood pressure, provoked by ligation of the aorta, caused overperfusion of the retina and breakdown of the blood retinal barrier: Bill et al.²⁶ confirmed this and demonstrated that sympathetic stimulation under conditions of acute arterial hypertension prevented retinal overperfusion.

There are therefore, indications that regulation of the retinal blood flow in response to altered perfusion pressure, may be brought about by both myogenic and neurogenic means. In the present study, though the diastolic blood pressure rise and retinal arteriolar constriction were contemporaneous (Fig. 3), there was no correlation between the magnitude of the blood pressure rise and the extent of the arteriolar constriction, suggesting that parallel but independent responses were occurring. Had the mechanism of constriction been solely myogenic, then a degree of correlation between blood pressure and retinal vessel response might be expected.

This study has demonstrated a significant association between retinal vessel calibre and systemic autonomic nervous stimulation. The exact role of the autonomic nervous system remains to be defined, however the present results suggest that autonomic effects are a significant integral feature in the autoregulation of retinal blood flow.

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