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MECHANISMS OF BLOOD FLOW INCREASE INDUCED BY ELECTRICAL STIMULATION

S. HAJIZADEH, Ph.D., A. KHOSHBATEN,* Ph.D., AND A. ASGARI,* Ph.D.

*From the Dept. of Physiology, School of Medical Sciences, Tarbiat Modarres University, and the *Dept. of Physiology and Biophysics, Baghiatollah University of Medical Sciences, Tehran, I.R. Iran.*

ABSTRACT

Previous studies have shown that electrical stimulation (ES) increases blood flow, but the exact mechanisms are not clear. The present study was designed to clarify some of the underlying mechanisms involved in this phenomenon. White adult rabbits 4-6 months old were used. Animals were anesthetized by sodium pentobarbital (40 mg/kg, IV) and skin blood flow in the thoracic back was recorded using a Laser Doppler flowmeter (LDF). Square waves (20 Hz frequency, 0.5 ms duration, 15 V strength) were applied through a pair of surface electrodes placed on the skin of animals. Drugs were applied to the skin close to the tip of the laser optic probe. The results obtained were as follows: 1. Electrical stimulation increases local blood flow. 2. Blood flow increases dose-dependently by administration of substance P (SP). 3. The response of ES on local blood flow was augmented in the presence of SP. 4. SP-antagonists did not have any effect on basal local blood flow. 5. The effect of ES was attenuated in the presence of SP-antagonists, but this was not statistically significant. 6. Local blood flow increased in reserpinized animals. 7. The electrically-induced increase in blood flow in reserpinized animals was not statistically different from that of non-reserpinized animals. Based on the above findings, it could be concluded that release of vasodilating compounds such as SP from sensory nerve endings may contribute in electrical stimulation and increase blood flow.

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Keywords: Substance P, Blood flow, Electrical stimulation.

INTRODUCTION

Blood flow is an important factor which can affect the repair and healing of injured tissues. Changes in blood flow occur under the influence of different factors. There is some evidence that electrical stimulation (ES) increases blood flow locally.[2,6,12,13] It was also shown that different frequencies of low voltage electrical stimulation (LVS) have different effects on local blood flow.[6] On the other hand, Walker and his colleagues reported that high voltage electrical stimulation (HVS) has no effect on local blood flow.[15]

Mohr and colleagues using HVS have shown that increase in blood flow was more pronounced at 20 Hz frequency than other frequencies.[12] Both ES of C-afferents[14] and retrograde stimulation of sensory afferent fibers increase blood flow in related tissues.[9] Another study indicated that direct stimulation of the saphenous nerve increased blood

flow and edema formation in the rat hind-limb.[4] Release of calcitonin-gene related peptide (CGRP) has also been reported during transcutaneous electrical nerve stimulation.[10]

Based on current studies it is assumed that electrical stimulation of the skin increases local blood flow through two mechanisms, involving release of some vasodilator neurotransmitters from nerve endings and removal of sympathetic tone which result in an increase in local blood flow.[7]

Therefore, the aim of the present study was to investigate the role of sympathetic fibers and a vasodilator agent such as substance P (SP) on blood flow in response to ES.

MATERIALS AND METHODS

White adult rabbits 4-6 months old were used in this study (n=7 for each group). Animals were anesthetized by sodium pentobarbital (Sigma, USA) (40 mg/kg, IV). The skin on the dorsal aspect of the thoracic area was shaved and cleaned, and rubber surface electrodes were placed.

Laser Doppler flowmetry

Blood flow in the skin of the thoracic back was recorded using a laser Doppler flowmeter (MBF3D, Moore Instruments, U.K.) before and after treatment. In order to measure changes in blood flow due to ES and/or drug administration, the laser probe was stabilized in between the surface electrodes, through which ES was applied.

Drugs (Substance P and SP antagonist [-D-Pro^2, D-Phe^1, D-Trp^9], Sigma, USA) were administered (in different doses as indicated in the figures) as subcutaneous injections in the vicinity of the tip of the probe. Animals were reserpinized (Sigma, USA) (0.2 mg/kg for three consecutive days and 1 mg/kg four hours before the beginning of the recording experiment) to deplete the sympathetic nerve endings. Blood flow was monitored as flux, and changes in blood flow were expressed as changes relative to the average values recorded during the 3-minute period immediately before the use of ES stimulation or drug administration.

Electrical stimulation

ES of the skin was performed by application of carbon rubber surface electrodes on the shaved skin. Square pulses was applied with 0.5 msec pulse width, 20 Hz and 15 V strength. The results are expressed as mean ± SEM and a p-value less than 0.05 was considered statistically significant. Statistical data analysis was made by either paired or unpaired t-test or with ANOVA and Student's t-test.

RESULTS

Results indicated in Fig. 1 show that subcutaneous injection of SP increased blood flow locally in a dose-dependent manner. The blood flow increment induced by ES was augmented in the presence of SP (Fig. 2) and was statistically significant (p<0.01).

Although SP antagonist did not have any effect on skin local blood flow per se (Fig. 3A), pretreatment of animals

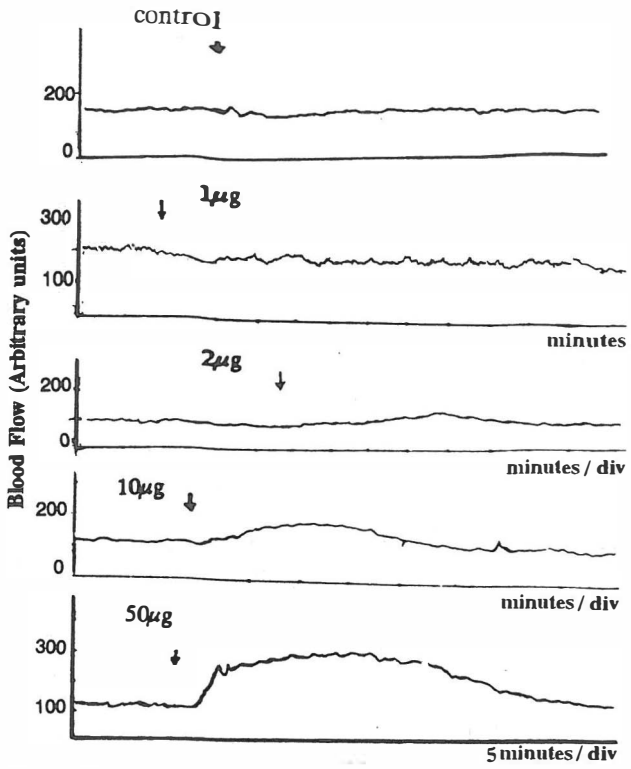

Fig. 1. The effect of different doses of substance P on local skin blood flow.

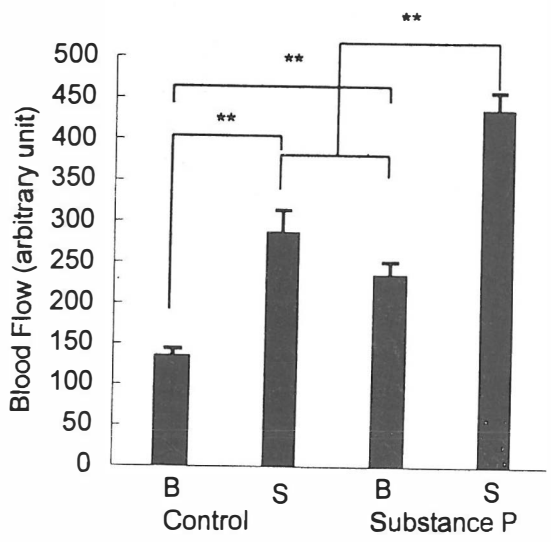

Fig. 2. The responses of skin blood flow to low voltage electrical stimulation in the presence and absence of substance P.**p<0.01, n=7, B= Basal, S= Stimulated.

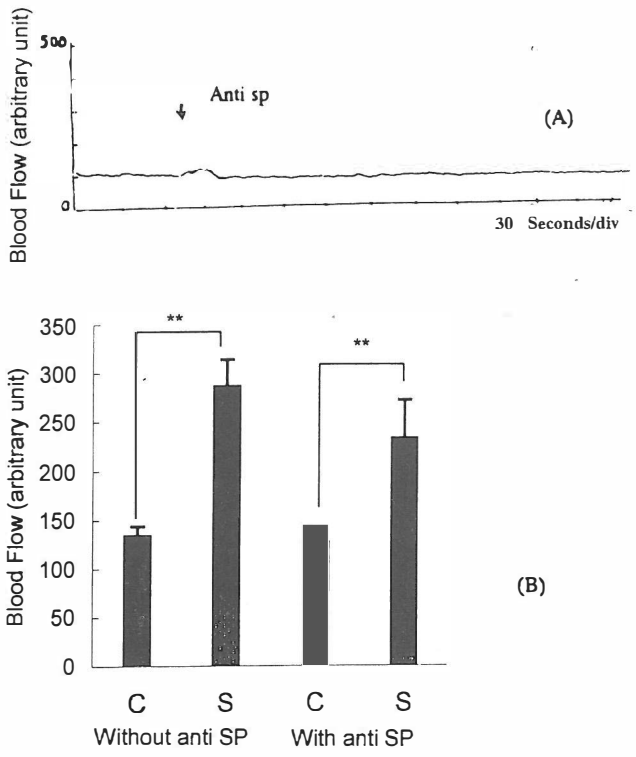

Fig. 3. The effect of substance P antagonist on local skin blood flow (A), and the response of skin blood flow to low voltage electrical stimulation in the presence and absence of substance P antagonist (B). $^{**}p<0.01$, n=7, C=Control, S=Stimulated.

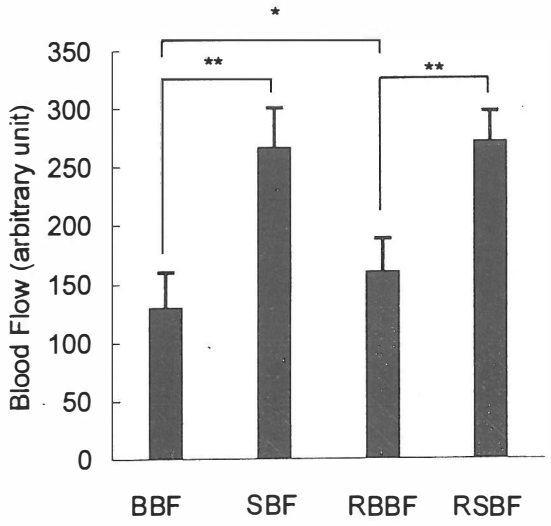

Fig. 4. Comparison between blood flow changes due to electrical stimulation in control (BBF, SBF) and reserpinized (RBBF and RSBF) animals. $^{*}p<0.05$, $^{**}p<0.01$, n=7.

BBF=Basal blood flow

SBF=Stimulated blood flow

RBBF=Reserpinized basal blood flow.

RSBF=Reserpinized stimulated blood flow.

by substance P antagonist partially inhibited the effect of ES on local blood flow (Fig. 3B). Basal blood flow increased in reserpinized animals significantly ($p<0.05$). Although ES caused blood flow to increase in reserpinized animals, this was not significantly different from intact animals (Fig. 4).

DISCUSSION

The present results revealed that ES increases local blood flow, both in the presence and absence of SP, but the increase in the presence of SP is significantly greater ($p<0.01$). SP produces vasodilation through smooth muscle relaxation by endothelium-dependent and independent manners.[3] Augmentation of blood flow increase by ES in the presence of SP is thought to be due to the release of SP or other vasodilator agents released from nerve endings. There is evidence that ES of C afferent fibers dilates blood vessels and that sympathetic fibers contain SP.[8,11] SP as well as excitatory amino-acids appear to be released in response to the stimulation of primary afferent C-fibers.[1] It appears that SP and ES have additive effects. According to our results, the idea that electrical stimulation of the skin may affect nerve endings and the release of some neurotransmitters which affect blood vessels is supported. This is in agreement with the findings of other investigators.[5,8]

The present data confirmed that SP antagonist did not affect local blood flow at rest, and it could not significantly inhibit the effect of ES on blood vessels. It indicated that SP does not contribute to resting blood flow[2] but may have a role during nerve stimulation. This is consistent with the findings of Stjarne and coworkers.[14] The partial reduction in vasodilation due to ES in the presence of SP antagonist may be because of SP receptor blockade. Carmody et al. reported that neither basal flow nor stimulus-evoked flow was significantly changed by topical administration of SP, or RP 67580-a NK_1 antagonist.[2] However, after treatment with reserpine which caused an increase in basal blood flow per se, there was no statistically significant difference in blood flow increase following ES in intact and reserpinized animals.

According to these findings and previous reports, it can be concluded that SP may be one of the mediators of blood flow increment following ES. Due to partial inhibition of the effect of ES by SP antagonist, it appears that whole blood flow increase induced by ES is not related solely to SP. As the results indicate, since ES had the same effect on reserpinized and intact animals, it could be concluded that sympathetic fibers do not have an effective role in the blood flow increase induced by ES in rabbit's skin, and SP may be one of the possible mechanisms involved in this phenomenon. To clarify the exact mechanisms of blood flow increase by ES, further investigation is required.

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