CORRELATION BETWEEN HEMODYNAMIC CHANGES AND MICROCIRCULATION IN THE RAT MESENTERY DURING ELECTRICAL STIMULATION OF THE CELIAC GANGLION

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The effect of stimulation of sympathetic vasoconstrictor fibers is mainly to limit the intramural blood flow through contraction of afferent arterioles. Slowing of the capillary blood flow and microvasomotor reactions can be observed during stimulation of different parts of the sympathetic nervous system in rats by intravital microscopy of the microcirculation of the mesentery or skeletal muscle [2, 4]. Meanwhile, if changes in the systemic arterial blood pressure (BP) due to stimulation are compared with the dynamics of microcirculatory processes, no complete correlation can be found in their development. For instance, maximal vasoconstriction arises after changes in BP have reached their maximum. The initial response of the microcirculatory system to stimulation of sympathetic fibers, moreover, takes the form of transient acceleration of the blood flow, and this is seen on biomicroscopy as translucency of the vessel. On the basis of these observations it has been suggested that electrical stimulation of the celiac ganglion in rats must lead, at least initially, to inclusion of the cardiac component in the general hemodynamic response and to an increase in the cardiac output. Morphological grounds for suggestions of this type exist, for we know that the celiac ganglion in rats contains not only vasomotor efferent fibers, but also afferent nerve fibers.

This paper describes the results of measurement of the cardiac output (minute volume) in Wistar rats during electrical stimulation of the celiac ganglion, and these results are analyzed by comparison with those obtained by the study of microcirculatory effects under the same experimental conditions.

EXPERIMENTAL METHODS

Two main series of experiments were performed. In series I (six rats, mean weight 276 g) hemodynamic parameters were determined. For anesthesia, Inactin from Promonta, West Germany) was injected intraperitoneally in a dose of 10 mg/100 g. Polyethylene catheters were introduced into the right carotid (PE 50) and right femoral (PE 50) arteries and into the right subclavian vein (PE 10). The animal's body temperature was maintained automatically at 37.5 ± 0.5°C by means of a bench with infrared heater. The systolic, diastolic, and mean BP were recorded in the right femoral artery by means of a two-channel recorder (HP/321, USA) and two transducers (MP 15, from Micron Instruments, USA). The cardiac output was measured by the dye dilution method [3]: 10 µl of a 0.3% solution of indocyanin green (Cardiogreen, USA) was injected through the subclavian vein into the right ventricle. Blood samples for determining the Cardiogreen concentration were taken continuously from the right carotid artery at a constant rate (1.1 ml/min), by means of a Perfusor IV pump (from Braun, West Germany). The blood was reinfused after determination of the cardiac output. The Cardiogreen concentration was determined in blood flowing through a miniature cuvette by a 25-µl densitometer. By means of a special absorption filter (length of passage 80 nm, from A. Schott, West Germany) changes in photic flux could be recorded. Changes in voltage of the photoelectric cell were recorded as dilution curves by means of a compensating recorder

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TABLE 1. Changes in CO, BP, TPR, and D 6 sec after Beginning of Stimulation of Celiac Ganglion (M ± m)

Exptl. series	Experimental conditions	ΔCO, ml·min^{-1}·100 g^{-1}	ΔBP, mm Hg	ΔTRP, mm Hg·ml^{-1}·min·100 g	ΔD, μ
I	Initial hypertension (n = 22); ΔBP_{max} = 15.45 ± 1.50 mm Hg* (time of maximal change of BP 5.39 ± 0.74 sec)	7,68±1,39* (23,4±4,9%)*	7,18±2,43‡ (7,06±2,45%)†	—0,36±0,13‡ (—10,47±3,85%)‡	— —
	Initial hypotension (n = 5); ΔBP_{max} = —1200 ± 3.39 mm Hg‡ (time of maximal change of BP 1.64 ± 0.03 sec)	—1,51±4,04 (—1,7±9,1%)	5,00±2,23 (4,5±2,1%)	0,25±0,24 (9,8±9,4%)	— —
II	Hypertension (n = 35): ΔBP_{max} = 28.79 ± 1.96 mm Hg*	— —	18,08±1,6* (18,85±2,18%)*	— —	—12,24±4,26** (4,1±1,62%)**

*P < 0.001.
†P < 0.01.
‡P < 0.05.

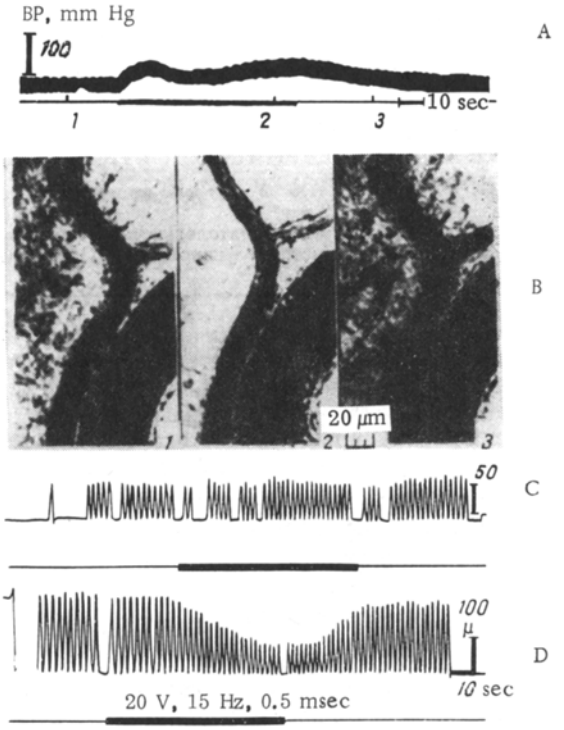

Fig. 1. Dynamics of changes in BP and microcirculatory network of rat mesentery during stimulation of celiac ganglion. A) BP recorded in carotid artery. Bold line indicates period of stimulation. Numbers correspond to times of filming; B) mesenteric microvessels; C, D) diameter of vessels recorded by image splitting method [1].

(Varian A 25, USA). Plasma ejection was calculated from dilution curves by the equations of Stewart and Hamilton (10 and 20 μl of Cardiogreen solution in 5 ml of human plasma were used for calibration). The cardiac output (CO) was then calculated from the plasma ejection volume, i.e., the minute volume (MV_{plasma}) and hematocrit index (Ht) by the equation: $CO = MV_{plasma}/(1 - 0.826\ Ht)$. The total peripheral resistance (TPR) was calculated from the mean BP and CO per 100 g body weight and expressed in units of resistance (in mm Hg^{-1}·min·100 g). The order of the experiments to measure CO was as follows: CO was measured in rats by the method described above 2 or 3 times with an interval of 5 min. The mean BP was recorded at

the time of injection of Cardiogreen before stimulation. The same hemodynamic parameters were determined during stimulation, when changes in cardiac output were studied in the first phase of the pressor reaction. After the end of stimulation, measurement of CO was repeated. Each rat was stimulated from 4 to 6 times. The experimental data were analyzed by the t test and paired t test and by regression analysis. In the experiments of series II microcirculatory changes in the animals (seven rats, mean weight 270 g) were studied during stimulation of the ganglion. Inactin (10 mg/100 g, intraperitoneally) or pentobarbital (5 mg/100 g, intramuscularly) were used for anesthesia. BP was recorded in the right femoral artery or the right carotid artery by catheters of the same type as in the experiments of series I.

The celiac ganglion was stimulated by a series of square pulses generated by a Disa-Multistim (Denmark) stimulator. The parameters of stimulation were: 10-30 V, 10-20 Hz, 0.5-0.7 msec. The stimuli were applied through a bipolar nichrome electrode implanted into the ganglion with the aid of MK-7 glue.

Observations of the microvessels were made with the MBI-15 (LOMO) microscope. Series of consecutive photographs were made of the blood vessels, from which subsequently the diameter of the microvessels was calculated and the microhemodynamic changes in the microvascular network were analyzed. To record changes in the diameter of the vessels periodically the image splitting method was used [1]. BP curves, image splitting curves, and marker of the period of stimulation were all recorded on a San-ei 142-8 (Japan) polygraph. The microfilming system was synchronized with the marker on the recorded curves.

EXPERIMENTAL RESULTS

The most typical response to stimlation, namely elevation of the systemic BP, was accompanied by distinct contraction of the relatively large mesenteric vessels (Fig. 1A) which, judging from their diameter, can be classed as resistive (200-400 μ). The blood flow in the lower regions of the microcirculatory stream gradually slowed down under these circumstances and, when the intensity of stimulation was high enough, the blood flow was almost completely arrested (Fig. 1B). The series of successive photomicrographs prepared with the same exposure, illustrated in Fig. 1, demonstrates the dynamics of the blood flow in the smallest mesenteric vessels. The diameter of these microvessels could remain unchanged during stimulation, as is also demonstrated by the results of recording the diameter by the image splitting method (Fig. 1C). The diameter of a large arteriole changed considerably in response to stimultion (Fig. 1D). The changes in the diameter of the microvessels are summarized in Table 1. The changes in the mesenteric microcirculation described above, if they appeared, were always of the same type regardless of the type of response of BP. As a rule, the photomicrographs were made 2-3 sec before stimulation, again in the phase of acceleration of the blood flow in the vessels, at the beginning of the slowing down process, at the time of maximal slowing or arrest of the blood flow, and after the end of stimulation, at the time of recovery of the normal circulation.

In the experiments of series II the initial data for the animals were as follows: BP 106.01 ± 4.99 mm Hg, mean diameter of vessels (D), 264.59 ± 14.5 μ.

In the experiments of series I the initial data for the animals were as follows: CO = 31.75 ± 1.739 $ml \cdot min^{-1} \cdot 100$ g, BP = 114.00 ± 4.94 mm Hg, and TPR = 3.578 ± 0.162 mm $Hg \cdot ml^{-1} \cdot min \cdot 100$ g. In the animals of this group, an initially hypertensive or initially hypotensive phase of the response could be distinguished on the basis of changes in BP. It is difficult as yet to state the reason why changes in BP of one or other type may appear, but the hypotensive response (initial phase) was found comparatively less frequently than initial hypertension and was evidently connected with the depth of anesthesia of the animal. Cardiac output was measured mainly in the hypertensive phase of the response. The results of these experiments are summarized in Table 1.

The results indicate a considerable (more than 20%) increase in CO in response to stimulation of the ganglion. Meanwhile TPR fell a little. As might be expected, positive correlation was found between the changes in TPR and the changes in BP in the first phase of the period of stimulation of the ganglion ($r = 0.4339$, $P < 0.05$) and the regression equations in this case were of the following form: $\Delta TPR = 0.0243 \cdot \Delta BP - 0.5348$, $\Delta BP = 7.7495 \cdot \Delta TRP + 9.9745$. The coefficient of correlation between ΔCO and ΔBP was $r = -0.1061$, which rules out the presence of correlation between the rise in BP and increase in CO during the first 6 sec after the beginning of stimulation of the celiac ganglion. Significant correlation likewise

was not found between BP and changes in the diameter of the vessels (experiments of series II, $r = +0.32$, $P > 0.05$), although vasoconstriction amounted to about 4%. It can be concluded from these results that microcirculatory changes observed in the mesentery in the initial phase of ganglion stimulation are due, on the one hand, to the vasoconstriction of resistive vessels and, on the other hand, to an increase in CO. The increase in CO in this case is evidently nonspecific in character and may be due to gradual narrowing of the lumen of the mesenteric arteries bringing blood into the mesentery and exclusion of the corresponding regions of the mesentery and intestine from the circulatory system. A direct or indirect influence at the same time on the contractile characteristics of the heart muscle likewise cannot be ruled out. Subsequent changes in CO may have been due to a response of the hemodynamic system as a whole to changes in TPR. Definite correlation was found between the changes in BP in the phase of maximal hypertension, expressed as a percentage, and ΔD ($r = -0.46$, $P < 0.01$). This suggests that the changes in BP and the corresponding microhemodynamic changes in the microcirculatory network of the mesentery at this stage were due mainly to vasoconstriction of the resistive vessels of the mesentery.

Comparison of the results of the two series of experiments shows that vasoconstriction of the mesenteric vessels in response to stimulation of the celiac ganglion took place against the background of a fall in TPR and, conversely, the initial hypotension was associated with a very small rise in TPR. This suggests that the vessels of other organs, such as the liver or spleen, must dilate in response to electrical stimulation of the celiac ganglion.

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EFFECT OF TETANUS TOXIN ON MECHANISMS OF REGULATION OF TRANSMITTER SECRETION IN NEUROMUSCULAR JUNCTIONS BY CALCIUM IONS

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The peripheral synaptic defect caused by tetanus toxin (TT) is due to a disturbance of transmitter secretion in the neuromuscular junction: spontaneous liberation of transmitter and, in particular, that induced by a nervous impulse, are inhibited [1, 4, 9, 12]. The most important factor regulating the secretion process is calcium ions [10, 11]. The stages of this type of regulation can be investigated by using various procedures: 1) changes in the calcium ion concentration gradient on the membrane whose permeability is unchanged, i.e., by changing the extracellular calcium concentration; 2) increasing the quantity of free cytoplasmic calcium on account of its liberation from intracellular depots, from mitochondria for example; 3) by changing membrane permeability for calcium ions by activation of endogenous ionophores of calcium channels, as is the case during depolarization, or by addition of exogenous ionophores; 4) by influencing the calcium-dependent process of secretion of quanta of transmitter through specialized regions of the presynaptic membrane ("active zones") directly.

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