Vasomotor escape from adrenaline, noradrenaline or acetylcholine in the dog hind limb

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Summary. The noradrenaline, adrenaline and acetylcholine-induced vasoregulatory escape was demonstrated in the vascular bed of intact or skinned and denervated dog's hind limb. Escape effect disappeared or decreased markedly under elevation tissue pressure in the examined hind limb. These data indicate that tissue pressure factor may take part in the mechanism of the escape phenomenon.

A number of investigations were devoted to exploring the nature of the disability of the vascular bed to maintain sustained level of response to pharmacological or sympathetical stimulation. This phenomenon was termed by Folkow[1] the autoregulatory escape. So far the mechanism of escape remains unclear. Relatively few studies have been concerned with the existence of the escape phenomenon in the vascular bed of the skeletal muscles. Conclusions resulting from Kjellmer's[2] investigation on escape in this region of circulation suggest that the chief mechanism of this phenomenon might differ from the one occurring in mesenteric vascular bed.

The present experiments were performed in order to examine the behaviour of the hind limb vascular bed during prolonged infusion of adrenaline (A), noradrenaline (NA) or acetylcholine (ACh). Another purpose of the investigations reported here was to compare the participation of the skin and muscle circulation of the hind limb in the creation of escape. Finally, the influence of increased tissue pressure upon the pattern of this phenomenon was also studied.

Material and methods. Experiments were performed on 37 mongrel dogs anaesthetized with sodium barbital 25 mg/kg. An isolated femoral artery was disected and its lumen was cannulated. Free ends of this artery were connected with a polyethylene tube. This tube was inserted into rotary pump which maintained flow at the constant level. Collateral circulation was interrupted in the experimental limb. Heparin was injected i.v. in a dose of 3 mg/kg. Among 24 tests, 12 were performed on the intact limb and the rest on the skinned and denervated one.

To investigate participation of the tissue pressure factor in the mechanism of the escape phenomenon, 12 experiments were performed with 2 pressure cuffs inflated to 20 mm Hg below control level of the perfusion pressure and applied around hind limb under study.

The following hemodynamic indices were measured continuously: systemic pressure, perfusion pressure, venous pressure and flow rate to the investigated limb with Satham pressure transducers and a square wave flowmeter probe (Hunter Instrument Co.). All measurements were recorded on the Sanborn Polygraph. A-chloride [Parke-Devis), NA (Levophed bititrate-Wintrop) and ACh-chloride (Metheson and Bell) were infused into the femoral artery at the constant rate of 5 µg/min for 10 min.

$$C = F/P_p \quad (1)$$

$$I_e = (C_3 - C_2)/(C_1 - C_2) \quad (2)$$

where:

F-flow in ml/min, P_p-perfusion pressure in mm Hg
C-conductance in ml min Hg^{-1}
C_1-initial conductance
C_2-maximal or minimal conductance during reaction,
C_3-maximal point of escape in terms of conductance.

Since ACh response has an opposite direction to the rest, its conductance data are signed with aminus mark.

Results and discussion. The first series of experiments was performed on the intact hind limb. This preparation may be considered to be dominated by 2 vascular beds located in skin and skeletal muscles. Results of this series of experiments showed that the vascular response to A, NA or ACh consist of 2 phase pattern: transient peak followed by escape effect in spite of continuous infusion of vasoactive compounds (table). The escape phase is most pronounced during ACh reaction ($I_e = 0.65 \pm 0.26$).

After skining and denervation of the hind limb, the overall pattern of responses to A, NA and ACh was not altered (upper pannels of figures). However I_e values for A and NA were nonsignificantly smaller, although the degree of escape from ACh was significantly larger. These data indicate that the vascular bed in the limb skeletal muscles is the

Effect of adrenaline, noradrenaline or acetylcholine on conductance in the vascular bed of the intact or skinned and denervated dog hind limb

Compound	Number of test	Intact hind limb	Skinned and denervated hind limb	Elevated external pressure (20% < P_p)	C_1	C_2	C_3	C_4	J_e
A	12	X			0.60±0.11	0.40±0.11	0.44±0.11	0.75±0.10	0.28±0.10
			X		0.52±0.07	0.35±0.03	0.38±0.04	0.75±0.10	0.23±0.04
				X	0.36±0.06	0.29±0.05	0.30±0.05	0.41±0.06	0.04±0.02*
NA	12	X			0.58±0.12	0.39±0.12	0.43±0.12	0.63±0.12	0.24±0.17
			X		0.55±0.07	0.33±0.03	0.37±0.03	0.56±0.06	0.19±0.02
				X	0.45±0.07	0.32±0.05	0.32±0.05	0.45±0.05	0.03±0.01*
AC	12	X			−0.57±0.12	−0.76±0.11	−0.64±0.12	−0.54±0.13	−0.65±0.26
			X		−0.50±0.05	−0.60±0.07	−0.52±0.06	−0.49±0.06	−0.86±0.04*
				X	−0.38±0.04	−0.47±0.06	−0.45±0.05	−0.37±0.04	−0.31±0.07*

Abbreviations: C_1, control conductance (ml mm Hg^{-1}); C_2, conductance at the peak point of vascular response; C_3, conductance immediately prior to with-drawal of infusion; C_4, conductance after the withdrawal of infusion; J_e, escape index. *Statistically significant differences (compared to control values) at $p < 0.05$ confidence level.

main source of the escape effect. The behaviour of escape from ACh in this part of the experiments is difficult to elucidate. It may be supposed that the decreasing basal tone after the denervation procedure/diminishes the peak phase ACh response, hence I_e is comparatively larger.

In the further part of presented experiments, vascular response to A, NA or ACh was examined in the skinned and denervated hind limb with increased tissue pressure. In thise case, the pattern of responses to tested compounds changed essentially. The typical examples of these responses are given in the figures (down pannels). Data from the table show lack of escape from A and NA, or of significant reduction from ACh.

Due to increased tissue pressure, the resistance in vascular bed of the hind limb becomes higher as a result constriction of small arteries and compression of thin-walled vessels[3,4]. In fact, it may be documented by a lower level of control conductance in this part of the experiments (table).

Accordingly to Whitesides' et al.[5] data, it may be assumed that the marked reduction of flow to the hind limb resulting from a higher resistance begins at the tissue pressure level which is 10–20 mm Hg lower than the diastolic pressure. To reach this level of perfusion pressure, applied cuff pressure was inflated up to 20% below perfusion pressure control values. Since these experiments were performed with constant flow, increased tissue pressure affected conductance, whereas flow still preserved. To our knowledge this experimental arrangement allows one to evaluate some aspects of the function of the tissue compartment in the creation of the escape phenomenon.

The results presented indicate directly that either the tissue pressure values or, indirectly, the capillary circulation my take part in the mechanism of escape. So far, Richardson and Johnson[6] reported that the mesentery capillary network is able to react with escape from catecholamine infusion. Cobbold et al.[7] found a close relationship between escape and C.F.C. values, in mesenteric circulation during sympathetic nerve stimulation. However, no similar reports

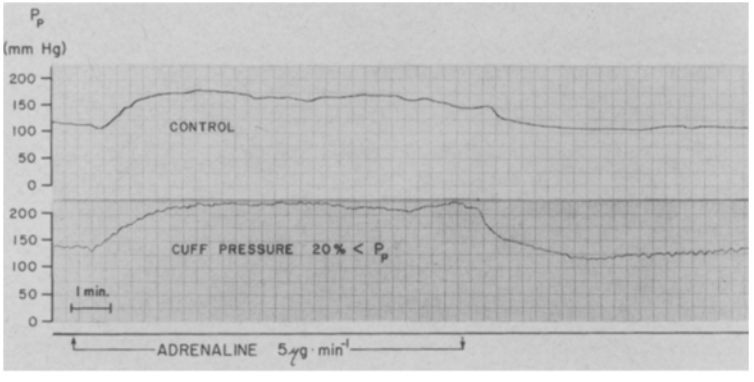

Fig. 1. Experimental record of perfusion pressure showing hemodynamic response to adrenalin infusion. Uppertrace response of the vascular bed in skinned, denervated dog hind limb. Lower trace: response under condition of higher external cuff pressure (20% < P_p) applied to perfusion limb.

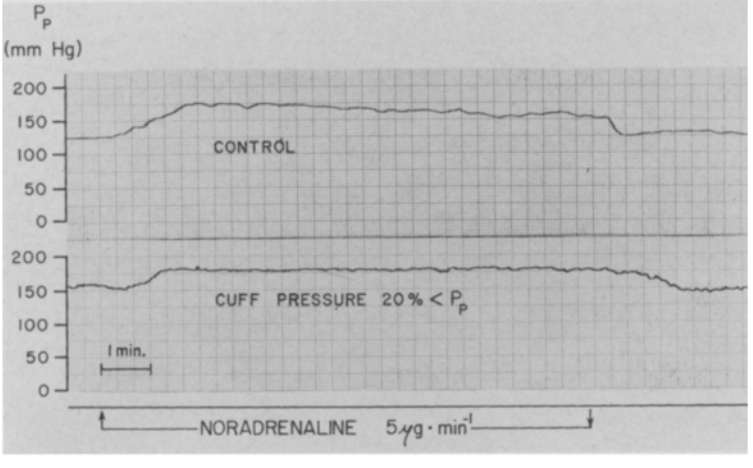

Fig. 2. Experimental record of perfusion pressure showing hemodynamic response during noradrenalin infusion. See legend in figure 1 for description.

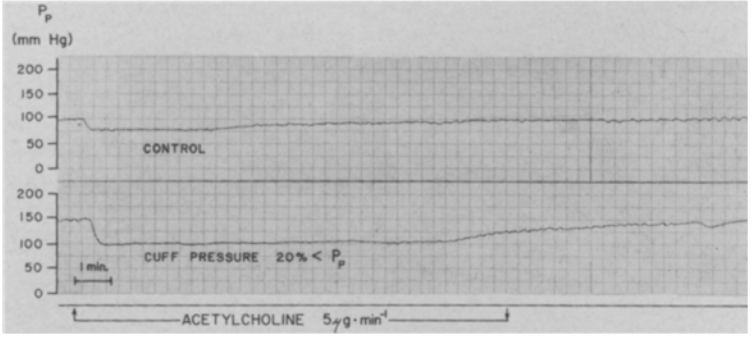

Fig. 3. Experimental record of perfusion pressure showing hemodynamic response during acetylcholine infusion. See legend in figure 1 for description.

concerning behaviour of capillary circulation in aspects of escape in the hind limb vascular bed have been found. Under the constant flow experimental procedure, increased tissue pressure may induce at least 2 shifts of flow in the hind limb: one of them is redistribution of the flow fractions. Since capillaries are most exposed to collapse due to higher extravascular pressure, the persisting flow could presumably open alternative channels at the time when capillaries are closed. However, the existence of shunts in the skeletal muscles of the hind leg remains a matter for dispute[8]. Then, it seems to be reasonable to consider another possibility, namely flow persistence through capillaries due to perfusion pressure elevation which balances higher tissue pressure. If this is a fact, then lack of escape could originate from detoriation of the fluid passage across capillary wall between the intra- and extravascular compartments.

In light of reports evidenced by Rodbard et al.[8] and Hinshaw et al.[10], the tissue pressure factor exerts an important influence on the pattern of flow regulation at the capillary level. Therefore disappearance or diminution of escape could result from 'stiffness' of the extravascular compartment which ceases to work in terms of the vasoregulatory function. Both hypotheses concerning the mechanism of escape changes under condition of higher tissue pressure indicate that the capillary level of circulation may be considered to be an important factor in the creation of this phenomenon.

Conclusion. During adrenaline, noradrenaline or acetylcholine infusion into the femoral artery at 5 µg/min for 10 min, a variable degree of escape from alocal typical response to tested compounds was observed either in intact or skinned and denervated dog's hind limb. Escape effect disappeared or markedly decreased under elevated tissue pressure in the hind limb. These results support the hypothesis that the tissue pressure factor and the component involved of peripheral blood flow regulation at the capillary leve could take part in the escape mechanism occuring in hind dog's leg vascular bed during the adrenaline, noradrenaline or acetylcholine prolonged infusion.

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Calcium transients in a molluscan smooth muscle

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Summary. The changes in myoplasmic calcium concentration during contraction were recorded in the anterior byssal retractor muscle of *Mytilus edulis* using murexide as calcium indicator, and were found to be qualitatively similar to those in striated muscles except for their slow time course.

It is well established that the contraction-relaxation cycle in muscle is regulated by changes in myoplasmic calcium concentration[2]. Though such changes (calcium transients) have been demonstrated in intact striated muscle fibres[3-9], no attempts have hitherto been made to record the calcium transients in various types of smooth muscle fibres. The present experiments were undertaken to study the calcium transients in the anterior byssal retractor muscle (ABRM) of a bivalve mollusc, *Mytilus edulis*, using murexide as a calcium indicator. *Material and methods.* The ABRM was dissected from the animal with a piece of shell attached to one end and the byssal organ left at the other, and teased in artificial sea water (ASW, 497 mM NaCl, 10 mM KCl, 20 mM $CaCl_2$, 52 mM $MgCl_2$, pH 7.2 by $NaHCO_3$) to obtain a fibre bundle of 1-1.5 mm diameter. The preparation was equilibrated in Ca-free ASW containing 4 mM murexide for 2-4 h to allow murexide to enter into the interior of the fibres. Then, the preparation was washed well with ASW, and mounted vertically in an acrylite chamber (4 × 3 × 1 cm thick) filled with ASW. The shell end was clamped, while the byssal end was connected to a strain gauge to record isometric tension at in situ fibre length. The preparation was stimulated with transversely applied sinusoidal a.c. current (100 Hz) through a pair of Ag plate electrodes. To obtain reproducible results, possible damage to the preparation during stimulation was avoided by the use of a.c. current which does not cause electrolysis[10], and by applying currents of submaximal strenght. No appreciable difference in the mechanical response was observed between the preparations treated with murexide and the untreated preparations. The calcium transients were recorded by means of a double-beam spectrophotometer

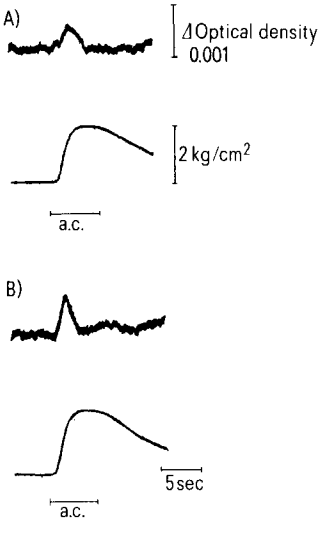

Fig. 1. Simultaneous recordings of the calcium transients (upper traces) and the isometric tension (lower traces). The preparation was stimulated with a.c. currents of varying intensity.