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ACETYLCHOLINE "TIGHTENS" PERIPHERAL CAPILLARIES INDEPENDENTLY OF PRESSURE EFFECTS

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ABSTRACT

We previously showed that acetylcholine (ACh) infused into the abdominal aorta of dogs at a rate of 127 μg ACh min^{-1} caused an increase in lumbar trunk lymph flow (L) of 35% while protein clearance into the lymph (LR) remained unchanged. These effects were accounted for by a 34% increase in reflection coefficient (σ) and a 54% increase in permeability-surface area product (PS). Since arterial pressure decreased, it was possible that the decrease in arterial pressure was responsible for observed changes. The current study was undertaken to test this possibility. Seven female dogs were anesthetized and prepared in the same manner as the previous study except that control abdominal aortic pressure was reduced with an aortic balloon to a mean of 81 mmHg. As ACh was infused, the balloon pressure was released so that the mean pressure for all dogs rose to 96 mmHg. The findings indicated that ACh produced a 24% increase in L ($P<.004$) while LR was unaffected. In a similar fashion to the results of the previous study, σ increased 43% ($P<.0000$) and PS rose 51% ($P<.008$).

These results clearly dissociate the effects of acetylcholine on permeability from any effects on arterial pressure and indicate a more direct effect of acetylcholine on the permeable segment. The results also suggest a general response of the capillary or postcapillary venule to vasodilation which restricts accession of protein into the interstitium during unloading of the vasculature by the process of edema formation.

In a recent study (1) we demonstrated that acetylcholine (ACh) when infused into the abdominal aorta of anesthetized dogs increased lumbar trunk lymph flow (L) without increasing lumbar trunk protein clearance (LR). These findings indicated a "tightening" of the hindquarter protein flux channels in the capillaries or perhaps postcapillary venules. The specific features were that reflection coefficient (σ) rose 34% and permeability-surface area product (PS) rose 54% indicating a proliferation of protein flux channels. These changes were associated with a rise in diffusive transport (D) by 101% and a rise in fractional diffusive transport from .20 to about .34. In these studies the recirculating ACh reduced arterial pressure (P_a) from a mean of 129 to about 92 mmHg. It was thus unclear whether these effects of ACh were directly attributable to the agent, or indirectly produced through the hypotensive effect of ACh. The present studies were designed to investigate whether capillary membrane tightening with ACh was related to its hypotensive effect or was independent of it. The results clearly show that ACh tightened capillaries without necessitating hypotension.

MATERIALS AND METHODS

These have been described in detail in the previous publication by Katz and Starr (1). Seven female conditioned mongrel dogs of weight (22.7 ± 1.2 SEM) kg were anesthetized with 30 mg/kg sodium pentobarbital, ventilated, and laparotomized to expose the lumbar trunk which was can-

nulated. The dogs were heparinized and infused with a sustaining bicarbonate saline solution (Na 138 mEq/l, K 8 mEq/l, HCO_3 28 mEq/l, and Cl 118 mEq/l) at 3 ml/min following an 8% loading infusion delivered at 50 ml/min. Pressures which were monitored were mean artery (P_a), right atrium, and hypogastric vein (P_v). Arterial blood gases were regulated with a volume cycle respirator. During the control period of 3 hours, a 0.9% NaCl infusion was delivered through the abdominal aorta during which time a Dotter-Lucas balloon catheter cranial to the infusion site was inflated to lower control P_a to approximately 80-85 mmHg. After 12 collections of lymph with midpoint plasma collections, fresh ACh bromide (65% ACh) in 0.9% NaCl was infused at a concentration of 1308 mg/dl and a rate of .15 ml/min delivering 127 μg/min of ACh to the hindquarters of the dog. During the experimental period which lasted up to 3 hours, the aortic balloon was deflated in order to bring abdominal aortic pressure at least to the control P_a and if possible somewhat higher than control. Lymph flow (L) and lymph/plasma protein concentration ratio (R) were used for computation by the fluctuation method described previously (1,2). Computed parameters or variables were solvent drag reflection coefficient (σ), permeability-surface area product (PS), diffusive or permeative transport which includes diffusion and vesicular transport (D=PS (1-R)), and fractional diffusive or permeative transport (FrD=D/LR). Interrelationships between parameters and variables of this study were examined by the paired Student t test, and comparisons to the previous studies in which P_a decreased were carried out by the non-paired t test. Probability values greater than .05 were taken as not significant (NS).

RESULTS

Table 1 shows the measured and computed variables and parameters for each dog during the control state and the experimental/control ratios for various times during the ACh infusion. Only a minority of all possible computations yield results for a number of reasons including random measurement error and an insensitivity of the method to generate σ and PS values when changes in LR are small. The comparisons with the earlier study in which P was allowed to decrease are shown at the bottom of the table and reveal that the control states of the dogs as defined by L, R, LR, P_v, σ, PS, D, and Fr were not different for each group despite a beginning P_a of 81 mmHg for the present group of animals and a beginning P_a of 129 mmHg for the previous group. Twenty-five measurements of experimental/control ratios were possible, and these were made between the intervals of 21 to 164 minutes following the beginning of ACh infusion.

Correlations were sought between experimental/control ratios and time, which in the case of the previous studies in which P_a decreased showed significant correlations between P_a and time and a negative correlation between R and time. In the current studies R was similarly negatively correlated to time ($R = -.5289$, $P = .0009$), but as in the former group, the relationship made only a maximum of a 3% change in R over the 30 minutes of any computation period. LR also showed a significant negative relationship to time ($R = -.4422$, $P = .0056$) resulting in a maximum of a 9% decrement in LR over any computational period. The analysis was independent of whether LR increased or decreased, and thus the finding of a time dependency of LR was not considered an important source of systematic error.

In Table 2, comparisons are made between the experimental/control ratios and unity for the current studies performed during increased P_a, and these are designated as the columns under the heading $P_a\uparrow$. The $P_a\uparrow$ columns are arranged on the right of the results of the previous study performed during conditions of decreased P_a which is designated $P_a\downarrow$ (1). Beneath each $P_a\downarrow$ or $P_a\uparrow$ column is the P value for the test of significance of the difference between each

Table 1

Control Values and Experimental/Control Ratios of Raw and Computed Data for ACh Infusion Studies With Pa Either Constant or Increased

Dog	L $\mu l \cdot min^{-1} \cdot kg^{-1}$	R	LR $\mu l \cdot min^{-1} \cdot kg^{-1}$	Pa mmHg	Pv mmHg	σ	PS $\mu l \cdot min^{-1} \cdot kg^{-1}$	D $\mu l \cdot min^{-1} \cdot kg^{-1}$	FrD
081982									
Control	20.29	.684	13.89	84	2.7	.342	6.13	1.94	.14
Ratios with Time									
112.5	1.00	.858	.86	.99	1.11	1.70	1.97	2.58	3.01
090282									
Control	20.98	.724	15.21	83	4.0	.349	11.52	5.79	38
Ratios with Time									
67.5	1.62	.982	1.59	1.12	1.00	1.37	2.80	1.61	1.01
82.5	1.74	.978	1.70	1.08	1.00	1.11	1.82	1.06	.62
90	1.73	.975	1.69	1.08	1.00	1.04	1.52	.89	.52
101282									
Control	22.20	.703	15.57	70	3.3	.306	4.52	1.35	.087
Ratios with Time									
22.5	1.69	.825	1.40	1.45	1.36	1.45	1.91	2.70	1.92
30	1.63	.861	1.39	1.43	1.36	1.80	4.58	6.08	4.36
52.5	1.30	.906	1.18	1.15	1.09	1.53	3.25	3.96	3.32
101482									
Control	8.58	.752	6.45	112	6.4	.300	3.91	.97	.15
Ratios with Time									
145	.36	.795	.28	1.06	1.15	1.74	.37	.59	2.13
160	.34	.789	.26	1.05	1.18	1.56	.22	.36	1.41
101982									
Control	31.24	.677	21.02	71	3.4	.351	9.72	3.23	.14
Ratios with Time									
21	1.88	.990	1.87	1.83	2.09	1.10	2.43	2.42	1.46
51	1.55	.916	1.40	1.37	1.33	1.13	1.08	1.23	1.02
58.5	1.56	.928	1.45	1.14	1.33	1.26	2.00	2.25	1.75
81	1.55	.936	1.46	1.10	1.51	1.13	1.40	1.54	1.19
103.5	1.38	.936	1.29	1.28	1.42	1.08	.81	.90	.73
118.5	.88	.928	.82	1.37	1.02	1.11	.64	.72	.99
141	.76	.926	.70	1.37	.80	1.35	1.18	1.32	2.12
148.5	.94	.926	.87	1.20	.98	1.12	.66	.74	.96
163.5	.96	.895	.87	1.30	.84	1.20	.78	.93	1.21
110282									
Control	28.87	.626	17.97	80	2.6	.396	7.06	2.60	.15
Ratios with Time									
67	1.10	.637	.70	.94	1.29	1.66	.98	1.60	2.26
74.5	1.11	.636	.71	1.02	1.29	1.65	.96	1.58	2.20
127	1.17	.615	.72	1.02	1.29	2.12	2.04	3.42	4.68
134.5	1.25	.605	.76	1.13	1.29	1.78	1.28	2.16	2.82
142	1.32	.585	.78	1.02	1.29	1.68	.88	1.52	1.94
110482									
Control	10.02	.726	7.28	68	3.8	.375	6.30	1.73	.24
Ratios with Time									
58	1.07	.861	.92	.97	1.40	1.22	.74	1.02	1.10
118	1.14	.802	.92	.96	1.36	1.88	1.51	2.30	2.50
Control Means	20.31	.699	13.91	81	3.8	.346	7.02	2.51	.18
SEM	3.24	.016	2.02	5.6	.5	.013	1.03	.62	.037
Control Means when Pa is decreased (Katz & Starr, 1983 Ref 1)	20.21	.616	11.79	129	4.6	.438	6.36	2.27	.20
SEM	3.04	.068	1.50	9.8	1.0	.067	.95	.37	.028
Itl (Pa↑ – Pa↓)*	.023	1.29	.819	4.38	.799	1.472	.468	.319	.365
P (Pa↑ – Pa↓)	NS	NS	NS	.0006	NS	NS	NS	NS	NS

*Absolute nonpaired t value for the difference between mean control value for the present studies carried out with constant or increased Pa (Pa↑), and our earlier study carried out at decreased Pa (Pa↓).

Table 2

Comparison of Experimental/Control Ratios of Transport Variables or Parameters (± 1 SEM) with a Mean Decrease (Pa↓) (Ref 1) or Mean Increase (Pa↑) in Arterial Pressure

	L		R		LR	
	Pa↓	Pa↑	Pa↓	Pa↑	Pa↓	Pa↑
Ratio	1.35 ± 0.18	1.24 ± 0.08	0.88 ± 0.03	0.84 ± 0.03	1.11 ± 0.10	1.06 ± 0.09
P1*	.029	.004	.0000	.0000	NS	NS
Itl (Pa↑ – Pa↓)†	.535		.962		.317	
P2**	NS		NS		NS	

	Pa		Pv		σ	
	Pa↓	Pa↑	Pa↓	Pa↑	Pa↓	Pa↑
Ratio	0.72 ± 0.03	1.18 ± 0.04	1.62 ± 0.25	1.23 ± 0.05	1.34 ± 0.10	1.43 ± 0.06
P1*	.0000	.0001	.010	.0001	.0007	.0000
Itl (Pa↑ – Pa↓)†	8.732		1.454		.798	
P2**	.0000		NS		NS	

	PS		D		FrD	
	Pa↓	Pa↑	Pa↓	Pa↑	Pa↓	Pa↑
Ratio	1.54 ± 0.30	1.51 ± 0.20	2.01 ± 0.42	1.82 ± 0.25	1.62 ± 0.26	1.82 ± 0.21
P1*	.042	.008	.012	.002	.007	.0002
Itl (Pa↑ – Pa↓)†	.077		.389		.588	
P2**	NS		NS		NS	

* P value for significance of difference between ratio and unity.
† Absolute nonpaired t value for difference between mean experimental control ratios for Pa↓ and Pa↑.
** P value for significance of difference between ratios for Pa↓ and Pa↑.

ratio and unity. At the bottom of each variable of parameter column is the non-paired t which tests the significance between the ratios for the $P_a\downarrow$ and $P_a\uparrow$ values. Immediately beneath the non-paired t value is the P value for the non-paired t test. It can be seen that except for the 28% decrease in P_a in the former group and an 18% increase in the current study, no differences in experimental/control ratios exist between this and the earlier study. In the current study, despite a significant 24% increase in L, LR was unchanged. P_v rose 23%, σ rose 43%, PS rose 51%, D rose 82%, and FrD went from .18 to.33.

DISCUSSION

We previously showed that ACh infusion into the hindquarters of the dog produced a rise in σ, PS, D, and FrD (1). Because ACh reduced P_a in these studies, it was possible that the experimental changes might be related to the changes in P_a rather than directly to ACh. The current studies clearly show, however, that σ, PS, D, and FrD increase with ACh regardless of whether P_a decreases. Moreover, the fractional changes in these parameters and variables are the same for both groups. This effect of ACh of "tightening" the protein flux channels while increasing their surface area remains poorly understood from a mechanistic point of view.

At this stage of understanding of the mechanistic interpretation of such phenomenologic membrane descriptions, the anatomic description of the action of ACh can only be speculative. One simple interpretation involves some direct action of ACh on the capillary or post-capillary venule which constricts the effective radius of an array of right circular cylindrical pores. Classic thermodynamic formulation links changes in σ to changes in PS such that a constriction raises and at the same time increases pore density which accounts for the elevation in PS (2).

An alternate proposal is that ACh increases PS by increasing endothelial vesicular turnover which is a possible mechanism for protein transport that is phenomenologically lumped into the PS descriptor (3,4). Thus if ACh has an action which primarily increases vesicular transport, and thereby increases PS, the mechanical relationship between PS and σ would secondarily raise the value for σ (2,5,6). However, there are a number of

theoretical obstacles to the concept of a vesicular shuttle including the facts that metabolic poisons do not adversely affect macromolecular uptake by them (7), that there does not seem to be an adequate supply of cellular energy available to provide the necessary impulse force to move the vesicle more than a few percent of its diameter (8), and that for at least the case of frog mesenteric capillaries, many vesicles which appear to be free in the endothelial cytoplasm are actually a part of an attached racemose structure opening either to the luminal or abluminal side (9).

A third possible mechanism of transfer of macromolecules across continuous capillary endothelia has been put forth by Curry, and colleagues (10,11) and may be subject to direct examination using current techniques. Curry proposes that the endocapillary layer which is a gel subtending the luminal and lateral surfaces of endothelial cells is a sophisticated but homogeneous discriminator composed of coils of proteoglycans crosslinked by albumin molecules. This fiber matrix can discriminate macromolecules by size, axial asymmetry, charge, concentration, and fluid flux. Since such a system can be examined in an artificial macroscopic experiment, the potential effects of ACh upon it may be testable.

Less interesting than the previous possibilities, but for the present equally feasible, is that ACh produces a shift in blood flow distribution through capillaries which individually possess a higher σ and surface area than that found in capillaries perfused in the control state. Perhaps this possibility could be examined by repeating the ACh infusions in a maximally perfused muscle dilated by exercise.

Whichever of these mechanisms or others is correct, the finding that ACh produces a tightening of the permeable segments of the vasculature independent of arterial pressure may be important in reducing protein efflux into the interstitium during edema forming states. It is possible that such a mechanism may be responsible for retarding further fluid flux by increasing transvascular oncotic gradient, for preventing some deleterious protein species from entering the interstitium, and for preventing a maldistribution of protein mass between the vascular and interstitial compartments.

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