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Effects of Acetylcholine on Peripheral Vascular Protein Permeability

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Abstract:

It is common practice to assume that when a vasodilator such as acetylcholine (ACh) produces a decrease in lymph/plasma protein ratio (R) while lymph flow (L) increases, permeability-surface area product (PS) and reflection coefficient (σ) are unchanged. However, if PS and σ are unaltered by a stimulus that increases L, then a decreased R can be associated with an elevated, constant, or reduced σ and PS. To test what the effect of ACh, a "pure vasodilator," is in the hindquarters of the anesthetized dog, we infused 127 μg ACh min^{-1} into the abdominal aorta of 6 female mongrel dogs while collecting lumbar trunk lymph in order to measure L and R. σ and PS were computed by the method of fluctuations over 15 min collections. The rise or decrease in LR was well correlated with L ($r=.951$) as expected, but was much less than predicted if σ and PS had been unaltered during ACh infusion. Computations indicated that σ rose with ACh approximately 34% ($P = .0007$) and PS rose 54% ($P = .042$) above control. This can be interpreted as a decrease in the radial dimension of the protein transport channels and an increase in the number of such channels per unit area, with both changes induced either by altered capillary anatomy or redistribution in a heteroporous system. Such an analysis seems compatible with the results of other studies in a variety of tissues which indicate that ACh tightens membranes either directly or through an effect mediated by reduced arterial pressure.

Whenever a "pure" vasodilator like acetylcholine (ACh) produces increased capillary filtration (J_v), the finding of an unchanged or reduced filtrate/plasma protein concentration ratio (R) is often accepted as evidence that protein permeability is unaffected (1-3). It can be shown, using irreversible thermodynamics, however, that as J_v rises and R decreases, that such a finding is a necessary but not a sufficient result to conclude that capillary permeability to protein is unaltered (4,5). It is the objective of this paper to change J_v by a vasodilator and assess whether capillary permeability to macromolecules changes or remains the same. The results strongly indicate that permeability characteristics of the canine hindquarters are altered by the infusion of ACh in such a way as to restrict protein accession into the interstitium as water flux increases.

Materials and Methods

Six female, conditioned mongrel dogs of weight (24.6±1.7 SEM) kg were anesthetized with 30 mg/kg sodium pentobarbital intravenously and were ventilated with a volume cycle respirator through a cuffed endotracheal tube to maintain an arterial pH of 7.4. Arterial, hypogastric venous, and subrenal lumbar trunk catheters were placed. The lumbar trunk drains the hind limbs, tail, pelvic wall, and a small fraction of the lower gut and genitourinary tract. The vast majority of such capillaries are of the continuous type (6). At the beginning of surgery, each animal received an 8% body weight load of 40°C solution consisting of Na 138 mEq/1, K 8 mEq/1, HCO_3 28 mEq/1, and C1 118 mEq/1 delivered at 50 ml/min. This was followed

by a 3ml/min infusion of the same solution modified by addition of sodium heparin and sodium pentobarbital to deliver 0.9 units/kg/min and 4 mg/kg/hr respectively. Surgery was completed in an hour followed by a 30 minute equilibration after which time lymph and plasma collections were begun. Mean arterial and hypogastric venous pressures were continuously monitored. Through an abdominal aortic catheter an infusion of 0.9% NaC1 was delivered at a rate of 0.15 ml/min. After a 3 hour control period of 12 collections of lymph with midpoint plasma collections, fresh acetylcholine bromide (65% ACh) in 0.9% NaC1 was infused at a concentration of 1308 mg/dl delivering 127 μg/min to the hindquarters of the dog. The experimental period lasted for another three hours.

Lymph flow was measured by timed collections in tared vessels and corrected for density. R was measured by the biuret method. The method of fluctuations shown and validated by Katz (5,7) was used to compute values for σ and PS for each data pair of L and R values separated by no more than one collection period. It has been shown that substantial errors in estimates of L and R about their steady state means lead to much smaller fractional errors in σ and to somewhat larger errors in PS which are generally within 20% of the true value (5). Only a minority of such calculations yield results for a variety of reasons including random measurement error and an insensitivity of the method to generate σ and PS values when changes in LR are small.

PS and σ are found by simultaneous solution of

$$PS = \frac{L\,(1-\sigma)}{\ln \frac{R_\sigma}{R-(1-\sigma)}}$$

at two different sets of L and R (8)

Diffusive transport is computed as $D = PS(1\text{-}R)$, and fractional diffusive or permeative transport is given by $FrD = D/LR$. Pearson product moment correlation coefficients (r) of σ, PS, R, L, LR, D, Fr D after ACh and arterial pressure (P_a), and venous pressure (P_v) versus time were computed. In addition, interrelationships between variables were examined. Results are expressed as means ± SEM and analyzed by the Student t test. A one tail test was used because expected changes in variables if any were predicted in advance. That is, increases were expected in L, LR, P_v; and decreases were predicted in R and P_a.

Results

Table 1 shows the summary of control values for L, R, LR, P_a, P_v, σ, PS, and FrD for the 6 animals and the values for these variables or parameters expressed as experimental/control ratios at various times in minutes after beginning the Ach infusion. Control values for L, R, LR, P_a, P_v were taken only during those control periods which yielded computable values for σ and PS. Thus only a small part of the three hours of control periods were used. For that reason, standard errors of the mean are left out since these are larger than the true variations over the control time. Moreover, individual values were not used to determine the group mean control values. This technique tends to increase the variance of the control values and thus the necessary differences between experimental and control results required for a given level of significance. There are 27 such ratios taken 9 to 165 minutes into the infusion. In order to increase the range of variation of P_a, one dog (080582) had the initial P_a reduced to 84 mm Hg by an abdominal aortic balloon. Responses of this dog were not outside the ranges for all variables for other dogs. Hence, its results were combined with the rest.

Only the two variables, R and P_a, showed a significant dependency upon

Table 1
Control Values and Experimental/Control Ratios of Raw and Computed Data for ACh Infusion Studies

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
030382									
Control	17.84	.849	15.14	147	3.1	.167	6.64	1.00	.07
Ratios with Time									
18.5	1.41	.974	1.38	.92	1.13	2.92	7.78	8.91	6.50
040782									
Control	12.31	.688	8.46	130	.6	.401	6.49	2.05	.23
Ratios with Time									
60	2.13	.769	1.64	.43	3.61	1.26	.95	1.42	.90
82.5	2.77	.725	2.01	.42	3.61	1.53	1.98	3.14	1.62
97.5	2.65	.677	1.79	.42	1.22	2.19	3.52	5.96	3.46
112.5	3.58	.676	2.42	.38	6.56	1.49	1.83	3.11	1.34
127.5	4.12	.555	2.29	.42	4.10	2.28	3.79	7.44	3.38
041482									
Control	27.01	.640	17.24	152	7.1	.424	10.49	3.80	.22
Ratios with Time									
90	1.27	.766	.98	.82	1.12	1.91	2.22	3.13	3.29
135	1.06	.731	.78	.69	1.12	1.38	.67	.99	1.31
041982									
Control	30.61	.351	10.73	132	4.6	.666	3.42	2.21	.21
Ratios with Time									
9	1.02	1.01	1.04	.78	1.05	1.02	1.40	1.40	1.36
22	.86	1.10	.95	.61	1.09	.99	1.48	1.41	1.50
29	1.20	1.06	1.28	.59	1.09	.96	1.08	1.05	.83
32	1.20	1.09	1.30	.64	1.12	.98	1.83	1.75	1.35
37	1.17	1.06	1.24	.59	1.12	.96	1.08	1.05	.85
042182									
Control	20.71	.534	11.05	127	6.7	.515	5.62	2.61	.23
Ratios with Time									
30	.92	.96	.88	.93	1.21	1.03	.83	.87	1.01
38	.96	.90	.86	.91	1.18	1.82	2.87	3.21	3.80
60	.74	.88	.66	.84	1.14	1.11	.44	.50	.77
68	.72	.90	.65	.86	1.18	1.11	.44	.49	.77
82	.59	.90	.53	.85	1.14	1.09	.42	.46	.90
90	.57	.91	.52	.87	1.18	1.09	.40	.44	.88
105	.72	.86	.62	.87	1.16	1.12	.56	.65	1.08
120	.66	.86	.57	.84	1.18	1.30	.92	1.06	1.91
135	.61	.86	.52	.83	1.21	1.12	.44	.51	1.01
158	.86	.82	.71	.79	1.16	1.17	.54	.66	.95
165	1.01	.85	.86	.79	1.21	1.55	2.01	2.36	2.81
080582									
Control*	12.78	.637	8.12	84	5.7	.458	5.50	1.97	.25
Ratios with Time									
90	1.20	.94	1.13	.75	.95	.93	.70	.78	.67
105	1.18	.94	1.11	.78	.95	.95	.79	.88	.76
113	1.22	.94	1.15	.71	.95	.92	.65	.72	.62
Control Mean	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
Coefficient of Variation (%)	36.9	26.9	31.1	18.6	53.0	37.2	36.6	40.3	33.7

*Pa artificially lowered with aortic balloon.

time. The Pearson product-moment correlation coefficient (r) of experimental/control ratio for R was −.6879, and the linear regression of the ratio as a function of time in minutes (t) was $-(.0018 \pm .0003)\ t + (1.02 \pm .03)$. The slope was different from zero at the $P < 10^{-4}$ level, and the intercept was indistinguishable from unity. Thus, over a maximum experimental time of 165 minutes, mean R decreased to .723 of its control value which is a mean change from .616 to .445. This effect may be related not only to ACh, but perhaps to volume expansion as well. Since values of R used to compute σ and PS are never separated by more than 30 minutes, any time dependent change in the R ratio due to progressive ACh or volume effects would be at most equal to 5% of the R ratio over that period. Hence, this time dependency was not considered to contribute a significant systematic error to the analysis.

The r value for the P_a ratio plotted against time was −.4032 which gave a linear dependency upon time of $-(.0015 \pm .0006)t + (.87 \pm .05)$. The slope is different from zero at P=.01, and the intercept was different from unity at P=.008. After 165 minutes the mean P_a decreased to 62% of its control value. This change may be related to progressive ACh effects or slow deterioration of the preparation. The cause is immaterial, however, because P_a is not used in any of the computations. It is recognized that reductions in P_a may produce a host of alterations in humoral and neuronal inputs and responses in the microcirculation which might influence the results. However, the predictability of such responses are not obvious since reductions in P_a are not correlated to changes in L.

If all the ACh ratios are meaned, and the mean ratios are tested for their differences from unity, the average effects of ACh can be presented as a set of mean ratios which is shown in Table 2. In 16 determinations, L rose, and in 11 it decreased slightly, so that the mean L rose 35% (P=.029). Despite this rise, mean LR was unchanged from control. P_a decreased 28% ($P \times 10^{-4}$), and P_v rose 62% (P=.010). Computed values for σ rose 40% (P=.0007) and PS rose 54% with wide scatter (P=.042). Despite the relative constancy of LR during ACh, the components of transport changed their relative values away from bulk flow and toward diffusive or permeative (diffusive plus vesicular) transport.

Table 2

Post ACh Experimental/Control Ratios With Student t Test, H_o = Ratio Is 1 Df = 26

Variable or Parameter	Ratio	SEM	t	P
L	1.348	.175	1.983	.029
R	.879	.026	−4.592	.0000
LR	1.106	.102	1.044	NS
Pa	.716	.034	−8.453	.0000
Pv	1.620	.251	2.467	.010
σ	1.339	.095	3.555	.0007
PS	1.542	.301	1.802	.042
D	2.013	.420	2.411	.012
Fr*D*	1.690	.261	2.640	.007

Discussion

This study provides strong evidence that infusion of ACh into the abdominal aorta in dogs produces a change in capillary permeability of the hindquarters with a rise in σ and less significant rise in PS. This may at first be intuitively difficult to reconcile, since a rise in σ should retard protein transfer, and an increase in PS should augment it. At least one model of protein transport makes it possible to understand such changes in anatomic terms. If one considers that protein and water transport pathways are right circular cylindrical pores, and that the Hagen-Poiseuille equation governs water flux, then the rise in σ can be interpreted as a narrowing of the effective pore radius. With such a narrowing, the pore density and entire pore area rises (5). Thus, the rise in PS (and in hydraulic conductivity surface area product if it were measured) is due to a rise in S most likely due to recruitment of more surface area. These studies do not address whether ACh

directly induces this change, or whether some uncontrolled changes such as a decrease in P_a or a rise in P_v may mediate this alteration. Although experiments with ACh during constant P_a may resolve this issue (9), the decrease in P_a as a stimulus for a rise in σ and PS is unattractive, since a number of investigators have shown a direct relationship between mean hydrostatic capillary pressure and σ and PS (4,7,10,11). Anatomic interpretations are varied and include not only a change in capillary anatomy but a redistribution of transcapillary flux toward smaller pores in a heteroporous membrane (11).

Other investigators have tended to discount results with ACh which have indicated alterations in vascular transport characteristics. Lewis and Winsey (1) studied cat hindlimb lymph flow during increasing infusions of ACh. Since ACh rarely produced an increase in R with an increase in L, the effects on membrane transport characteristics were considered nil. A more thorough analysis of their data would be needed to make such a statement, since as we have shown absolute changes in R alone cannot be used to assess whether σ or PS have changed.

Marci and Cevario (2) stimulated the (cholinergic) ciliary ganglion of the enucleated-perfused cat eye and noted that capillary water and albumin flux both rose some 2.7-fold. Since both rose by the same amount, the authors concluded there was no change in capillary permeability, which we have shown in the introduction to be untenable.

Carter, Joyner, and Renkin (12) used ACh as a "pure" vasodilator in the dog paw for comparison of effects of agents thought to increase capillary permeability with the presumption that ACh did not. In doses of about 5μg/min into the dog paw, they showed that while L_2/L_1 rose to 2.7, R_2/R_1 did not vary significantly from unity for albumin (radius = 35.5 A), α globulins (radius = 30 A), or macroproteins (radius 200 A). Their results were not analyzed in terms of σ and PS, but the raw data would seem to lead to the conclusion that σ was relatively unchanged because selectivity was unaltered. However, their calculations for PS which assumed σ equal to unity showed an increase in this number, probably due to a rise in S. It is known that S for water filtration increases when ACh acts with constant P_a rather than the reduced P_a seen in the present studies (9), and perhaps this could account for the apparent discrepancy between our results and those of Carter, Joyner, and Renkin. However, since the current study shows that PS rises with reduced P_a, it seems more likely that the causes of the discrepancy lie elsewhere, such as in the preparation, the dose, or in the assumption of their study that all protein flux is diffusive; that is, $\sigma = 1$.

A unique study by Kawana and Katori (3) examined infiltration of the interstitium of the skin by pontamine sky blue after graded intravenous doses of ACh in rats. They noted the striking finding that "...the amounts of extruded dye after intradermal injection of acetylcholine (up to 30mg/0.1ml) were less than those after saline control..." which agrees with the present study.

Studies by Grega, *et. al.* (13) detected no increased flux of macromolecules when ACh was infused into the canine forelimb. Although this is in accord with the current study, comparisons are difficult since L_2/L_1 did not change in Grega's studies.

Finally, a recent study of a number of vasodilators by Miller, Joshua, and Anderson (14) investigated protein leakage in rat skeletal muscle using *in vivo* fluorescence microscopy. Unlike many intravital studies which depend upon counting numbers of gross vascular "leaky sites," these investigators quantitated fluorescent intensity in the tissue after application of varying doses of vasoactive agents. Although ACh was not studied, and the locations of interest in the interstitium examined were difficult to standardize, they showed that a "pure" vasodilator like isoproterenol produced no augmentation of

protein leakage. A reasonable interpretation is that despite vasodilation and a probable increase in L, the failure of LR to rise was likely due to a tightening of the membrane or a rise in σ. It remains to be shown if other "pure" vasodilators demonstrate the same findings, and whether the effects are dependent upon a reduced P_a. If the finding is a general one, it may indicate an inherent regulatory capability of the capillaries to prevent protein flux into the interstitium during times of unloading of a plethoric vasculature into the interstitium by the formation of edema.

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