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THE EFFECT OF AGE ON THE RESPONSES OF HUMAN ISOLATED ARTERIES AND VEINS TO NORADRENALINE

We were interested in the letter of Scott & Reid (1982), which reported that vascular α -adrenoceptor sensitivity was not altered with increasing age. We have recently reported studies on α -adrenoceptor mediated mechanisms in human isolated arteries and veins, obtained *post mortem* (Stevens & Moulds, 1980, 1981; Stevens *et al.*, 1981; Stevens & Moulds, 1982a, 1982b), and have also performed extensive studies investigating the antagonism of contractile effects of noradrenaline on arteries and veins by various vasodilating drugs (Lipe & Moulds, 1981, 1982). In these studies digital arteries and/or metacarpal veins were obtained from a total of 90 patients. Since the clinical details of the patients from whom the vessels were obtained were all recorded,

we are in a position to assess the effects of the age of the patients and the α -receptor mediated responses.

The arteries and veins were cut into spiral strips and studied as we have previously described (Jauernig & Moulds, 1978; Jauernig *et al.*, 1978). The maximal contractile response to 80 mM KCl, and then at least two cumulative concentration-effect curves to noradrenaline were obtained on each arterial or venous strip. If more than one strip was cut from the same vessel, the mean of the values for that vessel was used. The following values were calculated from the second noradrenaline concentration-effect curve: $E_{\max}$ (g)-maximum response to noradrenaline expressed in grams; $E_{\max}$ (% KCl)-the maximum response to noradrenaline expressed as a percentage

Table 1 Statistical analysis (using Spearman's rank correlation test and linear regression) of the relationship between age of patient, and the $E_{\max}$ for noradrenaline, the pD_2 for noradrenaline and the contractile response to KCl (80 mmol/l) in human arteries and veins.

Correlation	Tissue	Sex	r_s	r	n
Noradrenaline $E_{\max}$ (% KCl)	Artery	Male	0.372*	0.366*	35
		Female	0.260	0.218	25
	Vein	Male	0.157	0.170	36
		Female	0.093	0.103	16
Noradrenaline pD_2	Artery	Male	0.129	0.069	35
		Female	0.069	0.110	25
	Vein	Male	0.245	0.287	36
		Female	0.121	0.199	16
KCl $E_{\max}$ (g)	Artery	Male	-0.296	-0.385*	35
		Female	-0.084	-0.007	25
	Vein	Male	-0.337*	-0.282	36
		Female	-0.251	-0.197	16
Noradrenaline $E_{\max}$ (g)	Artery	Male	-0.083	-0.278	35
		Female	-0.098	-0.022	25
	Vein	Male	-0.246	-0.189	36
		Female	-0.135	-0.070	16

n = number of patients

* = $P < 0.05$ (Student's *t*-test)

r_s = Spearman correlation coefficient

r = correlation coefficient (linear regression)

Table 2 Responses of arteries and veins taken from patients in the upper and lower age groups. Values shown are mean $\pm$ s.e. mean

		Noradrenaline $E_{\max}$ (% KCl)	KCl $E_{\max}$ (g)	Noradrenaline $E_{\max}$ (g)	Noradrenaline pD_2
Arteries from					
Males					
Age ≤ 55 years	(n = 6)	111.5 $\pm$ 14.3	3.68 $\pm$ 0.70	3.67 $\pm$ 0.50	6.28 $\pm$ 0.07
≥ 75	(n = 9)	158.0 $\pm$ 10.1	1.99 $\pm$ 0.28	2.99 $\pm$ 0.40	6.54 $\pm$ 0.12
Females					
≤ 60	(n = 5)	148.2 $\pm$ 9.4	1.65 $\pm$ 0.28	2.61 $\pm$ 0.47	6.48 $\pm$ 0.11
≥ 75	(n = 9)	158.9 $\pm$ 6.2	1.65 $\pm$ 0.30	2.48 $\pm$ 0.38	6.60 $\pm$ 0.08
Veins from					
Males					
≤ 55	(n = 4)	127.9 $\pm$ 23.3	3.27 $\pm$ 0.87	3.53 $\pm$ 0.29	6.60 $\pm$ 0.18
≥ 75	(n = 7)	161.7 $\pm$ 14.1	1.53 $\pm$ 0.26	2.36 $\pm$ 0.33	6.89 $\pm$ 0.11
Females					
≤ 55	(n = 4)	135.6 $\pm$ 21.3	2.70 $\pm$ 0.61	3.21 $\pm$ 0.36	6.72 $\pm$ 0.19
≥ 75	(n = 6)	150.7 $\pm$ 16.4	1.92 $\pm$ 0.34	2.85 $\pm$ 0.49	6.88 $\pm$ 0.11

of the response to 80 mm KCl (KCl $E_{\max}$); and pD_2 -the negative logarithm of the concentration of noradrenaline which produced a half maximal response. Correlation co-efficients r and r_s were calculated using linear regression and Spearman's rank correlation test (Siegel, 1956) respectively. The statistical significance of r and r_s was evaluated by a Student's t -test (Kendall, 1948).

The age of patients, both male and female, ranged from 35 to 89 years, but not surprisingly was biased towards the older age group. There was a significant positive correlation between patient age and the noradrenaline $E_{\max}$ (% KCl) for arteries obtained from male patients, but not for veins obtained from male patients, nor for arteries or veins obtained from female patients (Table 1). There was also a significant negative correlation between patient age and the KCl $E_{\max}$ for both arteries or veins obtained from males, but not for either arteries or veins obtained from females (Table 1). There was no significant correlation between patient age and noradrenaline $E_{\max}$ (g) or noradrenaline pD_2 for arteries or veins (Table 1). The mean values of the responses of arteries and veins from males and females in the upper and lower age groups are shown separately in Table 2.

These results are basically in agreement with those of Scott & Reid (1982) in that in arteries and veins from males and females, both the strength of the

contractile response to noradrenaline and the sensitivity to noradrenaline are unaltered with increasing age. Our results demonstrate, however, that in both arteries and veins obtained from males, the contractile response to KCl is greater in the lower age group and then declines with increasing age, whereas in arteries and veins obtained from females the contractile response to KCl in the lower age group is smaller than in males, but remains constant with increasing age. The increase in noradrenaline $E_{\max}$ (% KCl) with increasing age which we have observed in the arteries obtained from males is, therefore, most likely a result of expressing the noradrenaline response as a percentage of the response to 80 mm KCl.

We, therefore, agree with the conclusions of Scott & Reid (1982) that α -adrenoceptors in this biological system do not change with increasing age.

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CALCULATION OF DRUG CONCENTRATION IN PLASMA AFTER EQUILIBRIUM DIALYSIS

We wish to comment on the methods used to calculate the final concentration of drug in plasma after equilibrium dialysis.

The technique of equilibrium dialysis is used frequently to determine the binding of drugs to plasma proteins. Because the drug distributes between buffer and plasma during dialysis, it is necessary to calculate the final concentration of drug in plasma after equilibrium ($C_{p_{\text{equ}}}$) has been reached in order to determine the plasma concentration associated with a particular unbound fraction, α . This is particularly important for drugs which exhibit concentration-dependent binding to plasma proteins, since α will change as the concentration on the plasma side of the membrane falls during dialysis. For such drugs, the *in vitro* measurement of α is often used to calculate the unbound concentration (C_{p_u}) of drug by the relationship $C_{p_u} = \alpha \times C_{p_{\text{equ}}}$. Thus, accurate determination of $C_{p_{\text{equ}}}$ is essential. For drugs not displaying concentration-dependent binding, direct determination of $C_{p_{\text{equ}}}$ is not necessary when radio-labelled drugs are employed, but is necessary when using direct chemical methods.

Although theoretically the most direct method to calculate $C_{p_{\text{equ}}}$ is to employ a specific chemical analytical technique (e.g., gas-liquid chromatography, high pressure liquid chromatography, or spectrophotometry), radiolabelled compounds of high specific activity are most commonly employed in binding studies. Radiochemical methodology has the advantage of being more sensitive, accurate and requiring much less time than conventional analytical procedures. Determination of α can be done simply by dividing the d/min per volume of buffer by the d/min in an equivalent volume of plasma after dialysis. An essential component when using radiochemical

drugs of high purity is to check their stability in the plasma and buffer of the dialysis system (Yacobi & Levy, 1975).

When radiolabelled compounds are used for binding studies, two alternative approaches are available to calculate $C_{p_{\text{equ}}}$: a mass balance method and a specific activity method. We wish to present evidence showing that the specific activity method is preferable to the mass balance method.

The equations for mass balance are as follows:

$$(1) C_{\text{init}} = C_{b_{\text{equ}}} + C_{p_{\text{equ}}}$$

$$(2) C_{b_{\text{equ}}} = \alpha \times C_{p_{\text{equ}}},$$

therefore $(3) C_{\text{init}} = \alpha \times C_{p_{\text{equ}}} + C_{p_{\text{equ}}},$

and $(4) C_{p_{\text{equ}}} = \frac{C_{\text{init}}}{1 + \alpha}$

The term C_{init} represents the total concentration of drug initially present in the dialysis system, including drug from both the plasma and buffer compartments. $C_{b_{\text{equ}}}$ represents the concentration in buffer after equilibrium. In order to add concentrations, this method assumes that (a) the volume of buffer is equal to the volume of plasma after dialysis and (b) there is complete recovery of drug in the plasma and buffer after dialysis. The first assumption is often incorrect because of differences in osmolalities between buffer and plasma. Therefore, during analysis, the volume of plasma increases while the volume of buffer decreases (Abel *et al.*, 1979). The second assumption is difficult to validate because many dialysis systems do not permit complete recovery of fluids, and therefore, measurement of plasma and buffer volumes after dialysis cannot be carried out. Moreover, binding to membranes or dialysis cells will produce incomplete recoveries.