well within one standard deviation of the overall mean Kp of $1{\cdot}71 \pm 0{\cdot}62 \times 10^{-3}$ cm h^{-1}. The mean Kp ($1{\cdot}33 \pm 0{\cdot}55 \times 10^{-3}$ cm h^{-1}) for water permeability through 6 specimens of skin which were not frozen before use is represented in Fig. 1 by the hatched bar. Student's *t*-test showed no significant difference between human skin permeability to water whether the skin had been frozen or not. The water Kp's obtained compare well with published values: Scheuplein (1971) quoted water permeability measurements through abdominal skin at 30 °C which give a Kp of $1{\cdot}2 \times 10^{-3}$ cm h^{-1} (when corrected for skin area), and the mean Kp for water permeability through human leg skin at ≈32 °C is $1{\cdot}3 \times 10^{-3}$ cm h^{-1} from work by Astley & Levine (1976).

In conclusion, the permeability of human post mortem skin to water (and probably other compounds for which water is a suitable model) is largely unaffected by freezing and thawing, even after prolonged freezing for up to 466 days. Also there is no trend to suggest that it will alter quickly at even longer times.

REFERENCES

Allenby, A. C., Fletcher, J., Schock, C., Tees, T. F. S. (1969) Br. J. Dermatol. 81 (Suppl. 4): 31–38

Astley, J. P., Levine, M. (1976) J. Pharm. Sci. 65: 210–216

Burch, G. E., Winsor, T. (1944) Arch. Int. Med. 74: 437–444

Cohen, J. L., Stoughton, R. B. (1974) J. Invest. Derm. 62: 507–509

Pitman, I. H., Rostas, S. J. (1982) J. Pharm. Sci. 71: 427–430

Scheuplein, R. J. (1965) J. Invest. Derm. 45: 334–345

Scheuplein, R. J., Blank, I. H. (1971) Physiol. Revs. 51: 702–747

Swarbrick, J., Lee, G., Brom, J. (1982) J. Invest. Derm. 78: 63–66

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Acetylcholine-induced inhibition of responses to field stimulation in rabbit pulmonary artery is unaffected by endothelium removal

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Acetylcholine (0·01–10 μmol $litre^{-1}$) relaxed normal rings (endothelium-retained) of rabbit pulmonary artery precontracted with clonidine (10 μmol $litre^{-1}$) while preparations with the endothelium removed responded with contraction only. Removal of the endothelium had no effect on contractions of the preparation to clonidine or field stimulation of the adventitial nerves (2 Hz, 10 s). Furthermore, the inhibitory effect of acetylcholine (0·3 and 1·0 μmol $litre^{-1}$) on contractions induced by field stimulation was not influenced by the vascular endothelium.

The ability of acetylcholine to diminish contractions induced by α-adrenoceptor agonists and several other drugs in isolated vascular preparations has recently been shown to be dependent on the presence of vascular endothelium (Furchgott & Zawadzki 1980; Chand & Altura 1981; De Mey & Vanhoutte 1981). It has been proposed that acetylcholine liberates a substance from the endothelium which in turn acts on the smooth muscle to mediate relaxation (Furchgott et al 1981). The release of this endothelial substance by acetylcholine is dependent on muscarinic cholinoceptor activation since the relaxant effect of acetylcholine is antagonized by atropine (Furchgott et al 1981). In vascular preparations in which the endothelium has been removed, acetylcholine produces only contraction, presumably by acting directly at muscarinic cholinoceptors on the smooth muscle cells (Vanhoutte 1974).

In addition to its direct and indirect effects on vascular smooth muscle, acetylcholine has been shown to inhibit sympathetic transmission in several blood vessels (Malik & Ling 1969; Rand & Varma 1970; Allen et al 1972). It is clear that this effect of acetylcholine is due to inhibition of transmitter release from the terminal noradrenergic varicosities and that the effect involves the activation of muscarinic receptors associated with the varicosities (Allen et al 1972; Steinsland et al 1973; Vanhoutte et al 1973). However, in view of the finding that the relaxant effect of acetylcholine on the vascular smooth muscle is mediated by a substance released from endothelial cells, it is possible that the inhibitory effect of acetylcholine on noradrenergic transmission might also be mediated or influenced by this substance. The present experiment was designed to investigate this possibility.

Methods and materials

Rabbits of either sex, 2 to 4 kg were killed by cervical dislocation and the main pulmonary artery was excised and trimmed of adhering connective tissue. Throughout, care was taken to avoid unintentional rubbing of the intimal surface of the preparation against foreign surfaces or itself. Rings of 0·5 cm length were cut from the artery and mounted vertically on two stainless steel hooks in a 15 ml organ bath, the upper hook being connected to either a tension or a displacement transducer. The bathing fluid was Krebs-Henseleit solution of the following composition (mmol $litre^{-1}$): NaCl, 119; KCl, 4·7; $NaHCO_3$, 2·5; $MgSO_4$, 0·45; KH_2PO_4, 1·0; $CaCl_2$, 2·5; D(+)-glucose, 11·1; EDTA, 0·067; ascorbic

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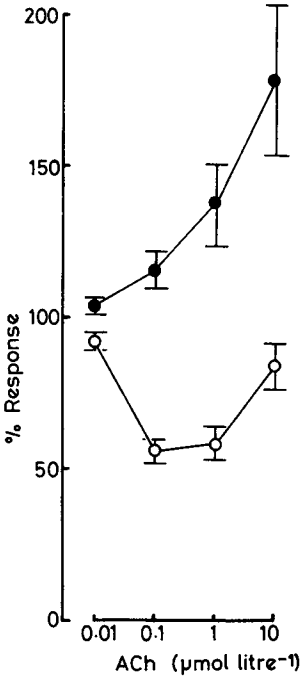

FIG. 1. The effect of acetylcholine (0·01–10 μmol litre^{-1}) on clonidine-contracted rings of rabbit pulmonary artery with (○——○) and without (●——●) endothelium. The effect of acetylcholine is expressed as the percentage change in the clonidine-maintained tension. Each point or bar represents a mean value from 5 to 11 experiments. Vertical bars represent standard errors of means.

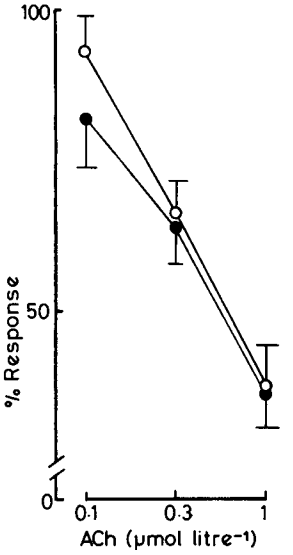

FIG. 2. The effect of acetylcholine (0·1–1·0 μmol litre^{-1}) on the responses of artery preparations with (○——○) and without (●——●) endothelium to sympathetic nerve stimulation. The stimulation-evoked responses in the presence of acetylcholine are expressed as a percentage of the control response to stimulation. Each point or bar represents a mean value from 5 experiments. Vertical bars represent standard errors of means.

acid, 0·14. The solution was maintained at 37 °C and gassed with 5% CO_2 in O_2. The artery preparation in the organ bath was located between two platinum electrodes to allow field stimulation of the adventitial sympathetic nerves.

The artery preparations were allowed to equilibrate for at least 2 h before commencing the experimental procedures. During this period the Krebs-Henseleit solution in the organ bath was replaced with a fresh solution at frequent intervals.

In experiments designed to investigate the effects of acetylcholine on vascular smooth muscle tone, the artery rings were maintained in a state of contraction by the addition of clonidine (10 μmol litre^{-1}) to the bathing solution. In this concentration clonidine produced its maximal contractile effect which was approximately 50% of the maximal contractile response to noradrenaline. In the presence of clonidine, the effects of acetylcholine added cumulatively (0·01–10 μmol litre^{-1}) were assessed on the state of contraction by isometric measurement. In experiments investigating the effects of acetylcholine on contractile responses to sympathetic nerve stimulation, responses to stimulation were obtained by applying 10 s trains of square wave pulses of 1 ms duration and supramaximal voltage (about 15 V cm^{-1}) at a frequency of 2 Hz, stimulation being repeated at 6 min intervals. The stimulation-evoked responses were approximately 30% of the maximum of the frequency response relationship which occurred with a stimulation frequency of 20 Hz. After stable responses to stimulation had been obtained, the effects of acetylcholine added cumulatively (0·1–1·0 μmol litre^{-1}) were assessed on the state of contraction by isotonic measurement.

In endothelium-denuded preparations, the endothelium was removed by rubbing the lumen of the artery ring with a roughened polyethylene tube before mounting it onto the tissue hooks.

Drugs used were: acetylcholine perchlorate (BDH) and clonidine hydrochloride (Boehringer Ingelheim).

Results have been expressed as the mean ± the standard error of the mean.

The unpaired two-tailed Student's *t*-test was used to test for significant differences between sample means. The pooled estimate of the two sampled variances was used to calculate the value of *t*. Probability levels of less than 0·05 were taken to indicate significant differences between sample means.

Results

The magnitude of isometric contractile responses to clonidine (10 μmol litre^{-1}) in normal (endothelium

present) and endothelium-denuded preparations, was not significantly different ($P > 0.05$); the contractions produced by clonidine in normal and endothelium-denuded preparations were 3·4 ± 0·3 g (n = 11) and 3·4 ± 0·5 g (n = 5), respectively. In normal artery preparations precontracted with clonidine, acetylcholine (0·01–10 μmol litre^{-1}) produced a concentration-dependent relaxation, with a contractile component occurring at high concentrations of acetylcholine. The maximal relaxation observed occurred with a concentration of 0·1 μmol litre^{-1} acetylcholine. In endothelium-denuded preparations, acetylcholine in the same concentrations produced only contraction. These results are summarized in Fig. 1.

Isotonic contractions to sympathetic field stimulation were not significantly different ($P > 0.05$) between normal and endothelium-denuded preparations; the contractions produced were 0·89 ± 0·21 mm (n = 5) and 0·82 ± 0·32 mm (n = 5), respectively. In normal and endothelium-denuded preparations, the stimulation-evoked responses were significantly reduced ($P < 0.05$) by acetylcholine in concentrations of 0·3 and 1·0 μmol litre^{-1}. In endothelium-denuded preparations, responses were also significantly reduced ($P < 0.5$) by a lower concentration of acetylcholine (0·1 μmol litre^{-1}). There was no significant difference ($P > 0.05$) in the degree of inhibition of the stimulation-evoked responses produced by acetylcholine between normal and endothelium-denuded preparations. These results are summarized in Fig. 2.

Discussion

The present finding that the response of rabbit pulmonary artery rings to acetylcholine is dependent on the presence of the vascular endothelium is in agreement with the observations of Furchgott & Zawadski (1980) in this and other arterial preparations. Typically our findings demonstrate that normal pulmonary artery preparations, precontracted with clonidine, responded to acetylcholine with relaxation, with a contractile component to the response becoming apparent at high concentrations of acetylcholine. In endothelium-denuded preparations also precontracted with clonidine, acetylcholine produced only further contraction. It is assumed that acetylcholine in low concentrations (up to 0·1 μmol litre^{-1}) selectively activates muscarinic receptors to release a substance from the endothelium which mediates smooth muscle relaxation (Furchgott et al 1981). At concentrations above 0·1 μmol litre^{-1}, it is assumed that the muscarinic cholinoceptors on the smooth muscle are activated to cause a contraction and consequently mask any relaxation.

In contrast to the effects of acetylcholine, the responses of the artery to clonidine or sympathetic stimulation were the same in normal and endothelium-denuded preparations. This suggests that the muscle relaxant is not released from the endothelium under these conditions or that the endothelium does not have a role either in the expression of the contractile response to clonidine or in noradrenergic neuroeffector transmission in this preparation. It is possible however, that the lack of influence of the endothelium on noradrenergic neuroeffector transmission may be due to the separation between the sympathetic nerves in the adventitia and the endothelium in the intima of the vascular tissue.

In contrast to the influence of the endothelium on the response of the vascular smooth muscle to acetylcholine, no evidence was obtained for an interaction between the endothelial substance released by acetylcholine and the inhibitory effect of acetylcholine on noradrenergic transmission, since the inhibitory effect of acetylcholine on stimulation-evoked responses was unaltered in endothelium-denuded preparations. Thus, at least in the rabbit pulmonary artery, muscarinic cholinoceptor inhibition of transmitter noradrenaline release is unlikely to be either mediated or modified by the vasoactive endothelial substance.

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REFERENCES

Allen, G. S., Glover, A. B., Rand, M. J., Story, D. F. (1972) Br. J. Pharmacol. 46: 527P–528P

Chand, N., Altura, B. M. (1981) Science 213: 1376–1379

De Mey, J. G., Vanhoutte, P. M. (1981) J. Physiol. 316: 347–355

Furchgott, R. F., Zawadzki, J. V. (1980) Nature (London) 288: 373–376

Furchgott, R. F., Zawadzki, J. V., Cherry, P. D. (1981) in: Vanhoutte, P. M., Leusen, I. (eds) Vasodilatation, Raven Press, New York, pp 49–66

Malik, K. U., Ling, G. M. (1969) Circ. Res. 25: 1–9

Rand, M. J., Varma, B. (1970) Br. J. Pharmacol. 38: 758–770

Steinsland, O. S., Furchgott, R. F., Kirpekar, S. M. (1973) J. Pharm. Exp. Ther. 184: 346–355

Vanhoutte, P. M., Lorenz, R. R., Tyce, G. M. (1973) Ibid. 185: 386–394

Vanhoutte, P. M. (1974) Circ. Res. 34: 317–326