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INTERACTION OF MONO- AND DIVALENT METALLIC CATIONS AND OF INDOMETHACIN ON THE MEMBRANE POTENTIAL OF VASCULAR ENDOTHELIAL CELLS *in vitro*

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1 Endothelial cells depolarized progressively when heated for 5 h at 45° C in the presence of calcium (1 mM), cupric (0.08 mM) or ferrous (0.04 mM) ions. In the absence of these ions, heating caused only slight depolarization. Higher concentrations of these ions caused depolarization even at normal body temperature (37° C).

2 Cuprous and potassium ions, although producing depolarization at 37° C, failed to augment the depolarization due to heating to 45° C.

3 Hydrogen peroxide caused depolarization which was potentiated by the presence of calcium, cupric or ferrous ions, but not by the presence of cuprous or potassium ions.

4 Indomethacin (0.25 mM) reduced the depolarization caused by calcium, cupric or ferrous ions at 37° C and also reduced the potentiation of heat-induced and hydrogen peroxide-induced depolarization which these divalent metallic cations produced. However, indomethacin failed to modify the depolarization caused by cuprous or potassium ions.

Introduction

An earlier study revealed that vascular endothelial cells possess a resting membrane potential which may be measured *in vitro* during exposure of the vessel wall to bathing fluids of known composition (Northover, 1975b). Heating the vessel wall to 45° C, or exposing it to histamine, depolarized the endothelial cells if the bathing fluid contained calcium ions but had no effect in the absence of calcium. Several anti-inflammatory drugs reduced the depolarization observed at 45° C in the presence of calcium. The experiments to be described in this paper were designed to explore further the inter-relationships between the effects of certain metallic cations, and of various types of physical and chemical injury, on the membrane potential of vascular endothelial cells.

Methods

Membrane potentials of endothelial cells from the thoracic aorta of guinea-pigs were recorded as described previously in detail (Northover, 1975b). The isolated aorta was cut longitudinally and opened out into a sheet which was then tied to the bottom of a superfusion trough. Glass micropipettes filled with 3 M KCl solution and having a resistance of approximately $10^7\ \Omega$ were lowered vertically by means of a micromanipulator towards the endothelial surface of the aorta. A recording was considered to begin with the first negative change of voltage encountered, and this was assumed to originate from the interior of the surface layer of cells. The pipette was allowed to remain within a cell for 50 s and then withdrawn into the superfusate. The difference in recorded voltage just before and just after withdrawal was taken as the membrane potential. The tip-potentials of the micropipettes were measured whenever the composition of the superfusate was altered, by the method of Adrian (1956). A micropipette was discarded and replaced by a new one if the tip-potential exceeded 10 mV. This problem was rare except when superfusates containing a low concentration of chloride ions were used. Except where otherwise stated, the experiments were conducted at 37° C in a basic superfusion fluid (BSF) of the following composition (mM): NaCl 130, KCl 6, glucose 11, $MgCl_2$ 2, tris-(hydroxymethyl)-methylamine 10, adjusted to pH 7.0 with acetic acid and oxygenated by a stream of air bubbles.

Results

Interaction between the effects of sodium, potassium, chloride, magnesium and calcium ions on the response to heating

Endothelial cells depolarized progressively during 5 h of heating to 45° C in a bathing fluid consisting of the BSF to which calcium chloride (1 mM) had been added (Table 1). Depolarization was very slight when the cells were heated in BSF to which the metal-chelating agent 1,2-bis-2-aminoethoxy-ethane-*NNN'N'*-tetraacetic acid (EGTA) had been added (Table 1). Exposure to a temperature of 37° C for 5 h caused no significant change in membrane potential in the absence or in the presence of calcium chloride (1 mM).

Replacement of all the NaCl in the BSF by an equiosmolar concentration of choline chloride (130 mM) or of sucrose (260 mM) increased the heat-induced depolarization observed in the presence of added calcium chloride but not in its absence (Table 1). Replacement of most of the chloride ions in the BSF by an equimolar concentration of the larger and less permeant benzenesulphonate ions had no significant effect on the magnitude of heat-induced depolarization either in the presence or in the absence of calcium ions (Table 1).

Omission of potassium or of magnesium ions from the bathing fluid or the addition of ouabain (10^{-4} M) to the BSF had no significant effect on the magnitude of heat-induced depolarization in the presence of calcium ions (Table 2).

Table 1 Effect of various ions on the membrane potential of endothelial cells during heating to 45° C for 5 hours

Composition of superfusate (mM)					*Membrane potential (mV negative)**					
Sodium chloride	*Sodium benzene sulphonate*	*Choline chloride*	*Sucrose*	*Calcium chloride†*	*At 37° C***	*During heating to 45° C. Duration (h) 1*	*2*	*3*	*4*	*5*
130	0	0	0	0	40 ± 5	40 ± 4	37 ± 6	38 ± 6	36 ± 5	35 ± 4§
130	0	0	0	1	36 ± 6	27 ± 6	25 ± 5	23 ± 6	18 ± 5	15 ± 4‡
0	130	0	0	0	38 ± 7	36 ± 6	35 ± 4	34 ± 6	35 ± 7	36 ± 5§
0	130	0	0	1	37 ± 6	30 ± 5	26 ± 4	22 ± 4	17 ± 4	14 ± 3
0	0	130	0	0	42 ± 5	39 ± 8	40 ± 6	39 ± 5	36 ± 5	38 ± 6§
0	0	130	0	1	30 ± 4	19 ± 4	18 ± 3	12 ± 3	9 ± 2	6 ± 2§
0	0	0	260	0	48 ± 6	42 ± 8	40 ± 6	39 ± 5	36 ± 5	37 ± 6§
0	0	0	260	1	31 ± 5	22 ± 4	16 ± 4	10 ± 3	7 ± 3	4 ± 1§

* The means of at least 30 observations ± s.e. mean. **Values recorded after 1 h exposure to a superfusate of the stated composition. †Where no calcium chloride was added, the BSF contained EGTA 0.1 mM.
‡ A significant difference exists between the value marked ‡ and values marked § (Student's *t* test, $P < 0.05$).

Table 2 Effect of substances which may alter sodium-pump mechanisms on the membrane potential of endothelial cells during heating to 45°C for 5 h in the presence of calcium chloride (1 mM)

Composition of superfusate (mM)			*Membrane potential (mV negative)**				
Ouabain	*Potassium chloride*	*Magnesium chloride*	*At 37° C***	*During heating to 45° C. Duration (h) 1*	*2*	*3*	*5*	*5*
0	6	2	36 ± 6§	27 ± 6	25 ± 5	23 ± 6	18 ± 5	15 ± 4†
0.1	6	2	33 ± 4π	29 ± 6	28 ± 6	26 ± 7	20 ± 5	14 ± 3‡
0	0	2	34 ± 7π	31 ± 7	30 ± 5	25 ± 6	19 ± 6	15 ± 5‡
0	6	0	39 ± 5π	26 ± 5	23 ± 4	21 ± 5	17 ± 4	13 ± 5‡

* The mean of at least 30 observations ± s.e. mean. ** Values recorded after exposure for 1 h to the stated conditions.
† No significant difference exists between the value marked † and the values marked ‡ (Student's *t* test, $P > 0.05$), or between the value marked § and the values marked π.

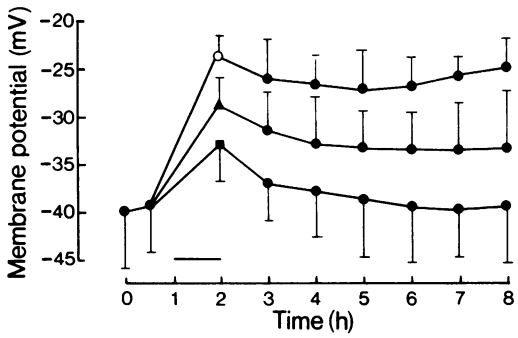

Figure 1 Membrane potential of endothelial cells in basic superfusion fluid (BSF) (•). The BSF contained added $CaCl_2$ for the period marked with a horizontal bar in a final concentration of 5 mM (▪), 10 mM (▲) or 20 mM (○). Each point is the mean of at least 30 observations. Vertical bars show s.e. mean.

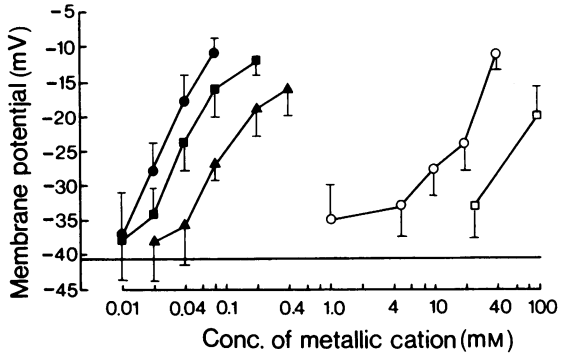

Figure 2 Membrane potential of endothelial cells exposed for 1 h to basic superfusion fluid (BSF) to which CuCl (•), $FeCl_2$ (▪), $CuCl_2$ (▲), $CaCl_2$ (○) or KCl (□) had been added. Each point is the mean of 30 observations. Vertical bars show s.e. mean. The horizontal line at −41 mV is the membrane potential of cells exposed to the normal BSF.

Comparison of the depolarizing influence of calcium, cuprous, cupric, ferrous and potassium ions

Calcium ions depolarized endothelial cells when added to BSF at 37°C (Figure 1). To varying extents, this effect was shown also by potassium, ferrous, cuprous and cupric ions (Figure 2). In view of the previously reported ability of indomethacin to reduce the depolarizing effect of calcium ions (Northover, 1975b), it was of interest to establish whether indomethacin would also reduce the depolarizing effect of these other metallic cations. Indomethacin (0.05 and 0.25 mM) failed to alter significantly the depolarizing effect of added potassium or cuprous ions but reduced the influence of exposure for 1 h to added cupric or ferrous ions (Figure 3).

Concentrations of calcium insufficient to cause significant depolarization at 37°C, nevertheless enhanced the depolarization caused by heating to 45°C (Table 3). In a similar way, the divalent cations of iron and copper, in concentrations which produced a slight depolarization at 37°C, enhanced the depolarization due to heating to 45°C (Table 3). In contrast, the monovalent

Table 3 Effect of various ions on the membrane potential of endothelial cells during heating to 45°C for 5 hours

Additions to the BSF				*Membrane potential (mV negative)**				
Metallic chloride	*Concentration (mM)*			*During heating to 45°C. Duration (h)*				
	Metallic chloride	*Indomethacin*	*At 37°C***	*1*	*2*	*3*	*4*	*5*
Control	–	0	41 ± 5	40 ± 5	41 ± 6	36 ± 5	37 ± 4	34 ± 6†
Calcium	1	0	35 ± 4	28 ± 5	22 ± 4	20 ± 3	16 ± 2	14 ± 3‡
Calcium	1	0.25	38 ± 5	39 ± 6	36 ± 5	31 ± 4	29 ± 4	28 ± 5
Potassium	24	0	34 ± 6	34 ± 5	33 ± 4	32 ± 5	30 ± 6	30 ± 5
Potassium	24	0.25	36 ± 5	32 ± 6	32 ± 5	33 ± 6	32 ± 5	34 ± 6
Cuprous	0.02	0	29 ± 5	30 ± 5	28 ± 5	26 ± 5	24 ± 4	25 ± 4
Cuprous	0.02	0.25	30 ± 5	28 ± 5	27 ± 6	25 ± 4	26 ± 4	26 ± 5
Cupric	0.08	0	27 ± 4	26 ± 5	20 ± 4	15 ± 3	13 ± 2	12 ± 3‡
Cupric	0.08	0.25	29 ± 5	29 ± 4	30 ± 6	31 ± 4	34 ± 5	33 ± 6
Ferrous	0.04	0	26 ± 4	20 ± 4	15 ± 3	15 ± 2	10 ± 3	11 ± 3‡
Ferrous	0.04	0.25	31 ± 6	30 ± 6	31 ± 5	28 ± 3	28 ± 5	29 ± 4

* The means of at least 30 observations ± s.e. mean. ** Values recorded after 1 h exposure to a superfusate of the stated composition.

† A significant difference exists between the value marked † and the values marked ‡ (Student's t test, $P < 0.05$).

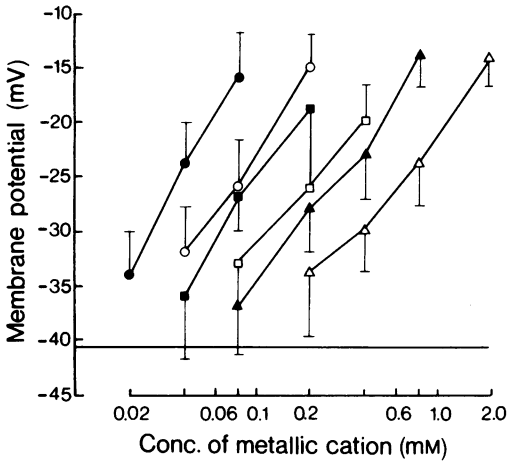

Figure 3 Membrane potential of endothelial cells exposed for 1 h to basic superfusion fluid (BSF) to which $FeCl_2$ (•), $CuCl_2$ (▪), $FeCl_2$ plus indomethacin 0.25 mM (▲), $FeCl_2$ plus indomethacin 0.05 mM (○), $CuCl_2$ plus indomethacin 0.25 mM (△), or $CuCl_2$ plus indomethacin 0.05 mM (□) had been added. Each point is the mean of 30 observations. Vertical bars show s.e. mean. The horizontal line at –41 mV is the membrane potential of cells exposed to the normal BSF.

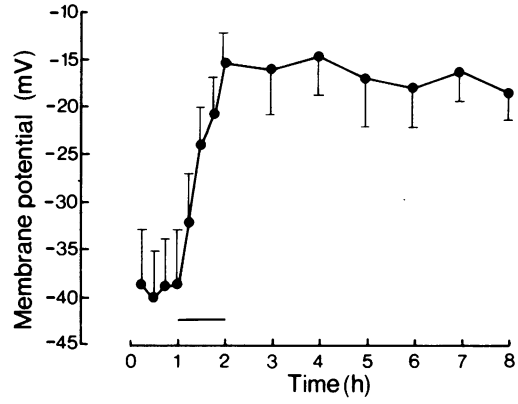

Figure 4 Membrane potential of endothelial cells. The basic superfusion fluid contained added hydrogen peroxide (120 mM) for the period marked with the horizontal bar. Each point is the mean of 30 observations. Vertical bars show s.e. mean.

Table 4 Interactions between various metallic ions and hydrogen peroxide on the membrane potential of endothelial cells

	Additions to the BSF			*Membrane potential (mV negative)**			
Metallic chloride	*Concentration (mM)*			*Duration of exposure to superfusate (min)*			
	Metallic chloride	*Hydrogen peroxide*	*Indo-methacin*	*15*	*30*	*45*	*60*
Control	–	0	0	39 ± 6	38 ± 5	41 ± 5	40 ± 5†
Control	–	30	0	36 ± 5	36 ± 7	34 ± 5	35 ± 4
Control	–	30	0.25	38 ± 5	37 ± 4	36 ± 6	34 ± 5
Control	–	120	0	32 ± 6	24 ± 5	19 ± 2	15 ± 4‡
Control	–	120	0.25	33 ± 6	22 ± 4	18 ± 4	14 ± 3‡
Calcium	1	0	0	34 ± 5	30 ± 6	32 ± 6	35 ± 7
Calcium	1	30	0	29 ± 5	30 ± 5	21 ± 4	15 ± 3‡
Calcium	1	30	0.25	36 ± 4	37 ± 5	35 ± 6	36 ± 6
Potassium	24	0	0	35 ± 7	36 ± 5	32 ± 6	33 ± 5
Potassium	24	30	0	34 ± 5	32 ± 4	33 ± 7	32 ± 4
Potassium	24	30	0.25	33 ± 6	30 ± 6	29 ± 3	31 ± 7
Cuprous	0.02	0	0	34 ± 6	30 ± 6	32 ± 5	28 ± 5
Cuprous	0.02	30	0	32 ± 7	31 ± 6	30 ± 6	27 ± 3
Cuprous	0.02	30	0.25	35 ± 7	34 ± 5	29 ± 5	26 ± 4
Cupric	0.08	0	0	30 ± 6	27 ± 5	24 ± 4	27 ± 4
Cupric	0.08	30	0	31 ± 5	23 ± 4	20 ± 3	11 ± 2‡
Cupric	0.08	30	0.25	35 ± 6	32 ± 5	30 ± 6	31 ± 6
Ferrous	0.04	0	0	34 ± 5	31 ± 5	28 ± 6	24 ± 4
Ferrous	0.04	30	0	28 ± 4	24 ± 4	17 ± 3	10 ± 4‡
Ferrous	0.04	30	0.25	36 ± 7	29 ± 4	33 ± 5	32 ± 6

* The means of at least 30 observations ± s.e. mean,

† A significant difference exists between the value marked † and the values marked ‡ (Student's *t* test, $P < 0.05$).

cations of copper and potassium failed to promote heat-induced depolarization (Table 3). Indomethacin protected against the enhancement of heat-induced depolarization caused by added calcium, ferrous and cupric ions (Table 3).

Interactions between the depolarizing effects of metallic cations and of hydrogen peroxide

Exposure of endothelial cells to various concentrations of hydrogen peroxide in BSF for 1 h at 37°C caused progressive depolarization, as shown in Table 4. Depolarization was only slightly reversed on returning the tissue to the original BSF after exposure to hydrogen peroxide (Figure 4). No reduction in the concentration of sodium chloride in the BSF was made to compensate for the addition of hydrogen peroxide. The permeability of endothelial cell membranes to hydrogen peroxide is not known and hence the extent to which the addition of this substance to the BSF induced an osmotic change across the plasma membrane of these cells is also not known. However, if an osmotic pressure was developed it is unlikely to have influenced the recorded membrane potential since the addition (100 mM) to the BSF of either lithium chloride or sodium chloride produced no significant change in membrane potential in an earlier study (Northover, 1975b).

The depolarization caused by hydrogen peroxide was augmented by the presence of calcium, cupric or ferrous ions but not by the presence of cuprous or potassium ions (Table 4). The combined effect of exposure for 1 h to hydrogen peroxide plus either cupric or ferrous ions was greater than the sum of the effects of these depolarizing agents separately (Table 4).

Indomethacin failed to reduce the depolarizing effect of hydrogen peroxide in BSF but reduced the larger depolarization caused by a combination of hydrogen peroxide with either calcium, cupric or ferrous ions (Table 4). Indomethacin reduced the effects of such combinations of depolarizing agents to that obtained with hydrogen peroxide in BSF. Indomethacin failed to modify the depolarizing effect of a combination of hydrogen peroxide with either cuprous or potassium ions (Table 4).

Discussion

The concentration of calcium ions normally present in mammalian extracellular fluids (approximately 1 mM) was sufficient to sensitize vascular endothelial cells to the depolarizing effect of heat or of hydrogen peroxide (Tables 1 & 4). In the case of heat-induced depolarization the effect of calcium ions was decreased by an opposing effect of the sodium ions (approximately 130 mM) present in most mammalian extracellular fluids and in the BSF (Table 1). This calcium-opposing effect of sodium ions was not shared by magnesium, potassium, choline, chloride or benzenesulphonate ions or by sucrose (Tables 1 & 2). Sodium ions compete with calcium ions for the occupation of binding sites in several tissues (Daly & Clark, 1921; Lüttgau & Niedergerke, 1958; Bohr, Seidel & Sobieski, 1969; Sitrin & Bohr, 1971). The ability of sodium ions to oppose the depolarizing effect of calcium ions on heated endothelial cells, therefore, is probably due to competition between the two cations for occupation of a cellular binding site which is important for the regulation of the membrane potential.

A lack of extracellular potassium or magnesium, or the presence of ouabain, impedes the function of the sodium-pump and leads to depolarization in several tissues (Casteels, 1966; Matthews & Sutter, 1967; Bolton, 1973). Depolarization did not occur, however, in the present experiments to any greater extent than in the BSF (Table 2). It is not known whether the membrane potential of these cells is independent of a sodium-pump mechanism or whether the pump is insensitive to ouabain and to a lack of potassium or magnesium.

Indomethacin reduced or prevented the depolarizing action of the divalent metallic cations but did not influence that of the monovalent metallic cations or of hydrogen peroxide. Furthermore, the ability of the divalent cations of calcium, copper and iron to potentiate heat-induced and hydrogen peroxide-induced depolarization was inhibited by indomethacin. It would appear, therefore, that indomethacin reduces the action of divalent but not of monovalent metallic cations on a component of the cell which is important for the regulation of membrane potential. Further work is required to identify more precisely the nature and location of this site.

It is not yet known whether the binding of naturally occurring endogenous divalent metallic cations, such as calcium, to cellular membranes promotes or permits the increases in the permeability of vascular walls which accompanies the inflammatory response. It is of interest, however, that several exogenously administered divalent metallic ions produce an increased permeability of the small blood vessels to plasma proteins (Miles & Wilhelm, 1955; Steele & Wilhelm, 1967; Mariano, Lourdes, Moraes & Neto, 1969). Endothelial cells exposed to histamine

exhibit several morphological and histochemical changes which have been shown recently to be augmented by the presence of calcium and to be inhibited by indomethacin (Northover, 1975a). The possibility clearly exists, therefore, that the anti-inflammatory action of indomethacin involves an effect of the drug on the binding of divalent metals to endothelial cell membranes.

References

ADRIAN, R.H. (1956). The effect of internal and external potassium concentration on the membrane potential of frog muscle. *J. Physiol.*, **133**, 631-658.

BOHR, D.F., SEIDEL, C. & SOBIESKI, J. (1969). Possible role of sodium-calcium pumps in tension development of vascular smooth muscle. *Microvascular Research*, **1**, 335-343.

BOLTON, T.B. (1973). Effects of electrogenic sodium pumping on the membrane potential of longitudinal smooth muscle from terminal ileum of guinea-pig. *J. Physiol.*, **228**, 693-712.

CASTEELS, R. (1966). The action of ouabain on the smooth muscle cells of the guinea-pig's taenia coli. *J. Physiol.*, **184**, 131-142.

DALY, I. de B. & CLARK, A.J. (1921). The action of ions upon the frog's heart. *J. Physiol.*, **54**, 367-383.

LUTTGAU, H.C. & NIEDERGERKE, R. (1958). The antagonism between Ca and Na ions on the frog's heart. *J. Physiol.*, **143**, 486-505.

MARIANO, M., LOURDES, B.M. de, MORAES, S. de & NETO, J.P. (1969). Cobalt ion action on the vascular permeability and mast cells of the rat. *J. Pharm. Pharmac.*, **21**, 709-710.

MATTHEWS, E.K. & SUTTER, M.C. (1967). Ouabain-induced changes in the contractile and electrical activity, potassium content, and responses to drugs, of smooth muscle cells. *Canad. J. Physiol. Pharmac.*, **45**, 509-520.

MILES, A.A. & WILHELM, D.L. (1955). Enzyme-like globulins from serum reproducing the vascular phenomena of inflammation. I. An activatable permeability factor and its inhibitor in guinea-pig serum. *Br. J. exp. Pathol.*, **36**, 71-81.

NORTHOVER, A.M. (1975a). Action of histamine on endothelial cells of guinea-pig isolated hepatic portal vein and its modification by indomethacin or removal of calcium. *Br. J. exp. Pathol.*, **56**, 52-61.

NORTHOVER, B.J. (1975b). Effect of anti-inflammatory drugs on the membrane potential of vascular endothelial cells *in vitro*. *Br. J. Pharmac.*, **53**, 113-120.

SITRIN, M.D. & BOHR, D.F. (1971). Ca and Na interaction in vascular smooth muscle contraction. *Am. J. Physiol.*, **220**, 1124-1128.

STEELE, R.H. & WILHELM, D.L. (1967). The inflammatory reaction in chemical injury. II. Vascular permeability changes and necrosis induced by intracutaneous injection of various chemicals. *Br. J. exp. Pathol.*, **48**, 592-607.

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