Br. J. Pharmac. (1975), 53, 113-120

EFFECT OF ANTI-INFLAMMATORY DRUGS ON THE MEMBRANE POTENTIAL OF VASCULAR ENDOTHELIAL CELLS *in vitro*

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1 Cells of the aortic endothelium isolated from the guinea-pig and bathed at 37° C with a calcium-free superfusion fluid had membrane potentials of −41 ± 7 mV (mean ± s.e. mean).

2 Depolarization was produced by addition of potassium (50-200 mM) or certain other monovalent metal cations to the superfusion fluid. Depolarization was rapidly reversed on return to the original superfusate.

3 Several divalent metal cations, notably calcium (16 mM), caused depolarization which was only slowly and incompletely reversed on return to the original calcium-free superfusate.

4 Repolarization after exposure to calcium was accelerated and made more complete by addition of indomethacin (0.25 mM) to the superfusate.

5 The trivalent cations of lanthanum, aluminium or iron (0.1 mM) inhibited the depolarizing effect of calcium (16 mM).

6 Exposure to histamine (100 μg/ml) or heating to 45° C for 1 h caused depolarization in the presence but not in the absence of calcium. Subsequent removal of histamine or cooling again to 37° C in the continued presence of calcium permitted only slow and partial repolarization. However, repolarization was more rapid and complete in the presence of indomethacin (0.25 mM).

7 Heating to 45° C for 5 h in the presence of calcium caused progressive and almost complete depolarization. Lanthanum, cinchocaine, indomethacin, flufenamic, meclofenamic and salicylic acids, phenylbutazone and aminopyrine each reduced the depolarization, but hydrocortisone, chloroquine, benzindamine, isoprenaline and aminophylline did not.

Introduction

Indomethacin and some pharmacologically related drugs reduce the uptake of calcium by electrically stimulated smooth muscle (Northover, 1972). Adenosine triphosphate-dependent binding of calcium to microsomes derived from smooth muscle or vascular endothelium is also inhibited by these drugs (Northover, 1973). Since the binding of calcium to cellular membranes alters their electrical properties (Bianchi, 1968; Bülbring and Tomita, 1970) the influence of anti-inflammatory drugs on the electrical activity of vascular endothelial cells was of interest. This paper describes the inter-relationships between the effects of anti-inflammatory drugs and of the concentration of calcium in the bathing fluid on the membrane potential of endothelial cells *in vitro*.

Methods

Albino guinea-pigs weighing 500-800 g were killed by a blow on the head. The thoracic aorta was removed immediately after death and opened longitudinally with fine scissors. A rectangular sheet of the vessel wall was tied, with its endothelial face upwards, to the bottom of a trough 10 cm long, 1 cm wide and 1 cm deep, made of Perspex. A thermostatically regulated water jacket around the trough enabled its contents to be kept at any selected temperature between ambient and 50° C (37° C unless otherwise specified). Superfusion fluid was warmed and delivered to one end of the trough at 10 ml/min by means of a roller pump. Fluid was withdrawn by suction through a constant level device from the other end. The contents of the trough were kept mixed by means of a stream of air bubbles. The composition of the basic superfusion fluid (BSF) was (mM): NaCl 130; KCl 6; glucose 11; $MgCl_2$ 2; tris-(hydroxymethyl)-methylamine 10, adjusted to pH 7.4 with acetic acid.

The mesothelium of the jejunal mesentery and the endothelium of the abdominal vena cava and

of the hepatic portal vein of the guinea-pig were used in place of the aorta in some experiments.

Membrane potentials were recorded by means of glass pipettes filled by boiling for 3 min at reduced pressure with freshly filtered 3 M KCl solution. Each pipette contained a Ag/AgCl wire electrode which was connected to a single-ended cathode follower circuit (Electrophysiological Instruments). The output from the cathode follower was led both to an oscilloscope and to a pen-recorder. No action potentials were seen on the oscilloscope screen throughout these experiments and all measurements of membrane potential were made from the pen-recorder trace.

One end of a glass tube filled with 3 M KCl solution gelled with 3% agar was dipped into the superfusate in the trough. The other end contained a Ag/AgCl wire electrode which was earthed via a 10^2 Ω resistor. At intervals throughout each experiment current was passed to earth through this resistor and the voltage generated between the superfusate and earth used to calibrate the recording and to determine the resistance of the pipettes. Pipettes were retained for further use only if their resistance continued to be approximately 10^7 Ω. In preliminary experiments the tip potential of 14 pipettes with this resistance was measured by the method of Adrian (1956) and found to be between 1 and 5 mV.

Pipettes were lowered vertically towards the tissue with a micromanipulator (Narishege, type MM3). A recording was considered to begin with the first negative change of voltage encountered, which was assumed to originate from the interior of a member of the surface layer of cells. The pipette was not moved for 50 s and was then withdrawn into the superfusate. The difference in voltage recorded just before and just after withdrawal was taken as the membrane potential. A recording was rejected as invalid only if the pipette failed to register the same potential (±1 mV) after withdrawal from the cell as it had done before entering it. No attempt was made to select cells with a particularly high or stable membrane potential. Membrane potentials registered at 10 places in the tissue were averaged and further groups of 10 such recordings taken at 15 or 60 min intervals throughout the experiment. To reduce vibration the superfusion trough and the micromanipulator were mounted on a heavy steel plate resting on 3 hollow rubber hemispheres.

Substances to be tested were freshly prepared as concentrated solutions which were then slowly delivered from a syringe-pump into the superfusate in the trough, the contents of which were kept mixed by a stream of air bubbles. Histamine dihydrochloride, acetylcholine bromide, (±)-isoprenaline sulphate, (−)-noradrenaline acid tartrate, cinchocaine hydrochloride, benzindamine hydrochloride and chloroquine hydrochloride were dissolved in distilled water and the solutions adjusted to pH 7.4 with NaOH solution. Concentrations refer to the free bases. Indomethacin, phenylbutazone, 1,2-bis-2-aminoethoxyethane-*NNN'N'*-tetraacetic acid (EGTA), phenylacetic acid, salicylic acid, flufenamic acid, meclofenamic acid and ibuprofen were dissolved in water with the aid of a slight excess of Na_2CO_3, and then rapidly adjusted to pH 7.4 with acetic acid solution. Concentrations refer to the free acids. Hydrocortisone sodium succinate was dissolved in distilled water to give a solution of pH 7.4. Concentration refers to the free alcohol. Aminophylline and aminopyrine were dissolved in distilled water and adjusted to pH 7.4 with acetic acid solution. The pH of the superfusate leaving the trough was recorded continuously to ensure that it remained at pH 7.4 ± 0.1.

Results

Aortic endothelium

Membrane potential in the presence of various metal cations The mean of 860 recordings of the membrane potential of cells in contact with the BSF was 41.1 ± 7.2 mV, with the inside negative. Addition of KCl to the superfusing fluid, with or without the addition of calcium 1 mM, caused a concentration-dependent depolarization (Figure 1). When the concentration of KCl was reduced to 6 mM again the cells promptly repolarized (Figure 2). Rubidium and caesium chlorides exerted similar actions to that of KCl, whereas the chlorides of sodium and lithium in concentrations up to 100 mM had no significant effect upon the membrane potential (Table 1). The tonicity of the bathing fluid was allowed to rise as a result of the addition of KCl. However, depolarization seen with KCl was presumably not due to the hypertonicity of the bathing fluid, since addition of similar concentrations of the chlorides of sodium and lithium failed to depolarize the cells.

Addition to the BSF of the chlorides of a number of divalent metal cations, including calcium, caused concentration-dependent depolarization (Table 1). In contrast to the effects of the monovalent metal cations the action of the divalent metal cations was only slowly and incompletely reversed by washing the tissue with the BSF (Figure 2 and Table 1). Reversal of the effect of calcium on returning to the BSF was accelerated by the presence of indomethacin (Figure 3). Magnesium was the only divalent metal ion tested which lacked a statistically significant depolarizing

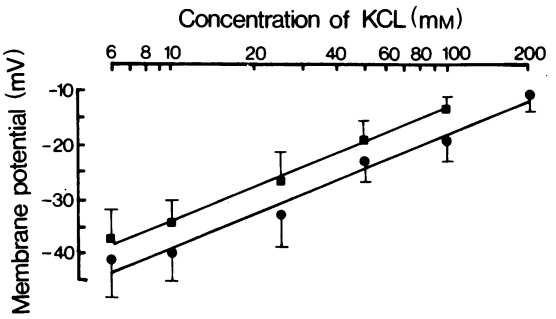

Figure 1 Membrane potential of aortic endothelial cells after exposure for 30 min to various concentrations of KCl in the absence of calcium (•) or in the presence of calcium 1 mM (▪). Each point is the mean of 40 observations. Vertical bars show s.e. mean.

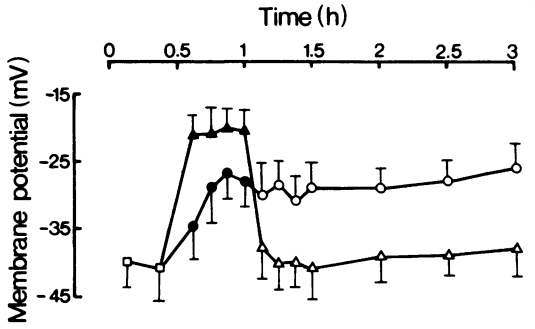

Figure 2 Membrane potential of aortic endothelial cells in the presence of 100 mM KCl (▲), 16 mM $CaCl_2$ (•) or the basic superfusion fluid (open symbols). Each point is the mean of 30 or 60 observations. Vertical bars show s.e. mean.

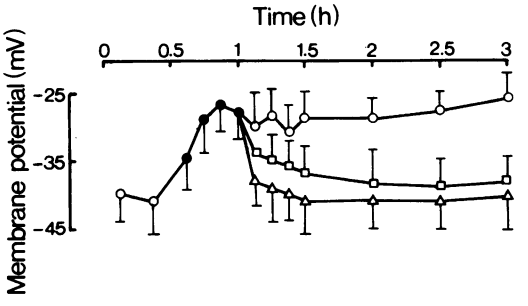

Figure 3 Membrane potential of aortic endothelial cells in the basic superfusion medium (○) or in the basic superfusion fluid plus 16 mM $CaCl_2$ (•), 0.25 mM indomethacin (□) or 0.1 mM $LaCl_3$ (△). In those experiments where indomethacin or lanthanum were added they were present in the superfusate from time 1 h onwards. Each point is the mean of 30 or 90 observations. Vertical bars show s.e. mean.

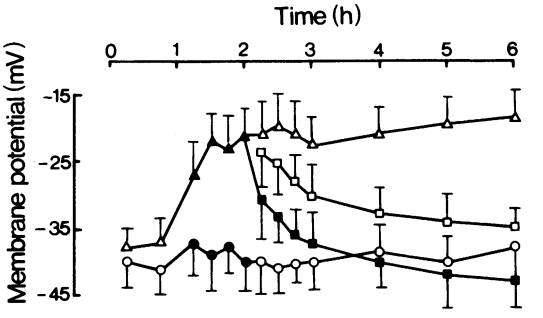

Figure 4 Membrane potential of aortic endothelial cells in the basic superfusion fluid (○ or •) or in a superfusate containing in addition 1 mM $CaCl_2$ (△ or ▲), 0.1 mM EGTA (▪) or 1.0 mM $CaCl_2$ plus 0.25 mM indomethacin (□). In those experiments where EGTA or indomethacin were added they were present in the superfusate from time 2 h onwards. Histamine (100 μg/ml) was infused from time 1 h to time 2 h (closed symbols). Each point is the mean of 30 or 90 observations. Vertical bars show s.e. mean.

action when added to the BSF in a final concentration of 16 mM (Table 1). It can be seen from Table 1 that the membrane potential of endothelial cells bathed with the BSF was not significantly different from that of cells bathed with a fluid containing no added magnesium. Moreover, the addition of magnesium 16 mM to a previously magnesium-free superfusion fluid caused no significant change in the recorded membrane potential of the endothelial cells whether or not the bathing fluid contained calcium (Table 1). Thus, magnesium not only lacked a calcium-like action but it also lacked a calcium-antagonizing action.

The chlorides of the trivalent metal cations of lanthanum, aluminium and iron failed to exert a statistically significant effect upon the membrane potential of endothelial cells when added to the BSF in a concentration of 0.1 mM, as shown in Table 1. Higher concentrations were not employed because they caused precipitation. Each of the three trivalent cations at a concentration of 0.1 M reduced the depolarizing effect of calcium 16 mM (Table 1). Like indomethacin, lanthanum accelerated the repolarization of cells which had been exposed to calcium previously (Figure 3).

Membrane potential in the presence of histamine Histamine rapidly depolarized endothelial cells in the presence but not in the absence

of calcium (Figure 4). The concentration of histamine required to produce a given degree of depolarization was reduced by increasing the concentration of calcium between 0.1 and 10 mM. Thus, histamine enhanced the depolarizing effect of calcium rather than causing depolarization itself. In the presence of calcium 1 mM neither acetylcholine nor noradrenaline altered the membrane potential when tested in concentrations up to 1 mg/ml.

Figure 4 shows that when the aorta was transferred, after treatment with histamine plus calcium, to a superfusate containing 0.1 mM EGTA but no added calcium, repolarization was rapid and complete. In contrast, discontinuation of histamine but continuation of the presence of calcium caused slow and incomplete repolarization. However, the endothelial cells repolarized after histamine had been discontinued and despite the continued presence of calcium, when indomethacin was added to the superfusate. Thus, the action of indomethacin in the presence of calcium resembles the effect of removing calcium with EGTA.

Table 1 Effect of various cations on the membrane potential of aortic endothelial cells

Cation†	*Concentration (mM)*	*Membrane potential (mV negative)**					
		During exposure to cation	*During washout of cation with the BSF. Duration (min)*				
			10	*20*	*30*	*40*	*50*	*60*
BSF	–	41 ± 6‡	40 ± 5	38 ± 5	42 ± 6	39 ± 4	40 ± 5	37 ± 4
BSF, magnesium-free §	–	39 ± 5	39 ± 6	37 ± 5	38 ± 5	36 ± 5	35 ± 6	34 ± 7
		Monovalent cations						
Potassium	100	20 ± 3π	29 ± 4	36 ± 5	40 ± 6	39 ± 6	41 ± 5	38 ± 7
Rubidium	100	22 ± 4π	31 ± 3	39 ± 5	37 ± 6	38 ± 5	42 ± 7	40 ± 5
Caesium	100	18 ± 3π	28 ± 4	35 ± 6	39 ± 5	36 ± 5	40 ± 6	42 ± 7
Lithium	100	37 ± 6	39 ± 7	41 ± 6	40 ± 7	40 ± 6	39 ± 8	41 ± 5
		Divalent cations						
Magnesium §	16	34 ± 6	38 ± 5	37 ± 7	39 ± 6	36 ± 5	39 ± 6	38 ± 6
Magnesium′	16	34 ± 7	35 ± 5	35 ± 4	32 ± 5	33 ± 5	32 ± 4	32 ± 4
Calcium	16	26 ± 4π	28 ± 3	29 ± 4	27 ± 5	25 ± 4	27 ± 5	26 ± 4
Calcium §	16	25 ± 5π	26 ± 5	25 ± 7	28 ± 4	24 ± 5	26 ± 3	25 ± 6
Calcium	8	28 ± 5π	29 ± 5	31 ± 4	32 ± 5	30 ± 6	29 ± 5	31 ± 4
Calcium	4	31 ± 6	32 ± 5	33 ± 5	30 ± 6	34 ± 5	31 ± 7	32 ± 5
Calcium	2	32 ± 5	32 ± 6	34 ± 6	35 ± 5	33 ± 6	36 ± 6	37 ± 7
Barium	16	27 ± 4π	26 ± 6	29 ± 5	31 ± 5	30 ± 6	31 ± 5	29 ± 6
Strontium	16	29 ± 4π	30 ± 4	28 ± 5	31 ± 6	30 ± 5	29 ± 4	28 ± 5
Manganese	16	21 ± 3π	18 ± 3	29 ± 4	32 ± 5	33 ± 4	35 ± 4	34 ± 5
Zinc	16	20 ± 3π	19 ± 4	25 ± 3	34 ± 5	36 ± 4	32 ± 5	35 ± 6
Cobalt	16	18 ± 4π	15 ± 3	17 ± 4	21 ± 3	22 ± 4	26 ± 5	26 ± 6
		Trivalent cations						
Lanthanum	0.1	43 ± 5	37 ± 6	38 ± 5	41 ± 6	42 ± 5	40 ± 7	41 ± 6
Aluminium	0.1	42 ± 6	40 ± 5	41 ± 6	43 ± 6	40 ± 7	39 ± 7	38 ± 6
Iron′	0.1	43 ± 5	36 ± 6	39 ± 5	40 ± 5	42 ± 6	40 ± 7	38 ± 5
Lanthanum + Calcium	0.1 + 16	39 ± 5	39 ± 6	42 ± 4	40 ± 7	39 ± 6	43 ± 5	40 ± 6
Aluminium + Calcium	0.1 + 16	38 ± 5	39 ± 7	38 ± 4	37 ± 5	39 ± 6	38 ± 4	37 ± 5
Iron + Calcium	0.1 + 16	34 ± 6	37 ± 5	39 ± 6	35 ± 5	35 ± 7	34 ± 4	35 ± 6

† The chloride salt of the cation added to the BSF for 30 minutes. § In these experiments the BSF was made up without $MgCl_2$. ′ In these experiments the BSF was made up without $MgCl_2$ but with $CaCl_2$ 2 mM added. * The mean of at least 30 observations ± s.e. mean.

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

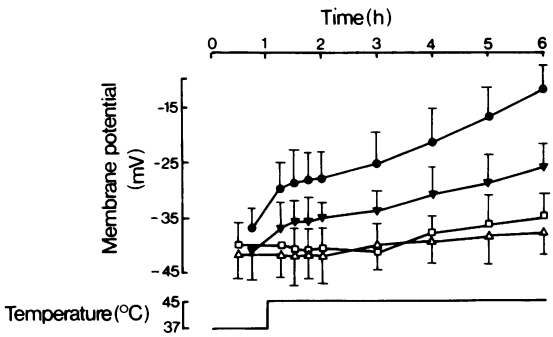

Figure 5 Membrane potential of aortic endothelial cells heated to 45° C. Potentials recorded in the basic superfusion fluid (▼) or in a superfusate containing in addition 1.0 mM $CaCl_2$ (●), 0.1 mM EGTA (■) or 1.0 mM $CaCl_2$ plus 0.1 mM $LaCl_3$ (△). Each point is the mean of 30 observations. Vertical bars show s.e. mean.

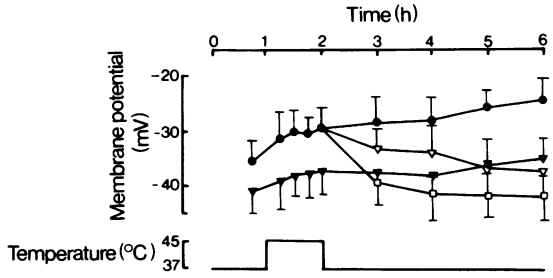

Figure 6 Membrane potential of aortic endothelial cells. The endothelium was heated to 45° C for 1 hour. Potentials were recorded in the basic superfusion fluid (▼) or in a superfusate containing in addition 1.0 mM $CaCl_2$ (●), 0.1 mM EGTA (■) or 1.0 mM $CaCl_2$ plus 0.25 mM indomethacin (□). In those experiments where EGTA or indomethacin were added they were present in the superfusate from time 2 h onwards. Each point is the mean of 30 or 90 observations. Vertical bars show s.e. mean.

Membrane potential at elevated temperatures Exposure of the endothelium to the BSF at 45° C for up to 5 h caused progressive depolarization which was enhanced by the addition of calcium 1 mM (Figure 5). Addition of EGTA 0.1 mM to the BSF protected against this depolarization (Figure 5). A temperature of 45° C was chosen for these experiments because preliminary experiments had shown that in the presence of calcium 1 mM lower temperatures caused only inconsistent depolarization. Heating to 50° C, on the other hand, caused depolarization more rapidly than at 45° C but the maximum depolarization produced was no greater than at 45° C.

In some experiments (Figure 6) aortas which had been heated to 45° C for 1 h in the presence of calcium 1 mM were cooled again to 37° C. In the continued presence of calcium the tissue remained depolarized, but when the tissue was exposed to the BSF some repolarization occurred. When the tissue was exposed after cooling to a superfusate containing EGTA the repolarization was almost complete (Figure 6). Even in the continued presence of calcium 1 mM the presence of indomethacin permitted considerable repolarization (Figure 6).

The depolarizing effect of prolonged exposure to a temperature of 45° C in the presence of calcium 1 mM was reduced by addition of lanthanum or cinchocaine to the superfusate (Figure 5 and Table 2). Anti-inflammatory drugs with a lanthanum-like action included indomethacin, ibuprofen, phenylbutazone and aminopyrine as well as flufenamic, meclofenamic and salicylic acids (Table 2). Phenylacetic acid, which is related to indomethacin chemically but which lacks anti-inflammatory activity (Northover, 1964; Durant, Smith, Spickett & Szarvasi, 1965) failed to prevent depolarization (Table 2). However, a number of drugs with well-established anti-inflammatory activity also failed to inhibit heat-induced depolarization. Thus, in the concentrations used, hydrocortisone, chloroquine, benzindamine, isoprenaline and aminophylline were inactive (Table 2).

Other tissues

Venous endothelial cells had membrane potentials similar to those of the aorta, whereas mesothelial cells had a lower potential (Table 3). The hepatic portal vein and the mesentery were both depolarized by the addition of calcium, an effect which was enhanced by histamine or by raising the temperature to 45° C (Table 3). The depolarizing effect of heating to 45° C for 5 h in the presence of calcium 1 mM was reduced by the addition of indomethacin 0.25 mM to the superfusate.

Discussion

In the present experiments endothelial cells from the aorta, the abdominal vena cava and the hepatic portal vein were found to possess similar membrane potentials. Few observations have been made previously of the electrical properties of vascular endothelium. Funaki (1961) recorded membrane potentials from endothelial cells of

Table 2 Effect of anti-inflammatory drugs and related compounds on the membrane potential of aortic endothelial cells during heating to 45°C for 5 h in the presence of 1 mM $CaCl_2$.

Drug	*Concentration (mg/100 ml)*	*Membrane potential (mV negative)**					
		At 37°C	*During heating to 45°C. Duration (h)*				
			1	*2*	*3*	*4*	*5*
Control	–	37 ± 6	28 ± 5	25 ± 6	21 ± 6	17 ± 5	13 ± 4†
Indomethacin	10	40 ± 5	34 ± 6	31 ± 4	30 ± 4	27 ± 5	25 ± 5‡
Indomethacin	5	38 ± 7	30 ± 5	28 ± 6	27 ± 5	22 ± 4	22 ± 6‡
Indomethacin	2	38 ± 6	29 ± 6	27 ± 4	22 ± 4	19 ± 5	16 ± 5
Cinchocaine	10	39 ± 7	36 ± 6	33 ± 5	30 ± 4	27 ± 4	26 ± 6‡
Cinchocaine	1	38 ± 5	35 ± 5	30 ± 5	29 ± 6	22 ± 6	19 ± 5
Phenylbutazone	40	38 ± 4	35 ± 7	31 ± 4	28 ± 4	25 ± 5	24 ± 3‡
Phenylbutazone	20	35 ± 5	31 ± 6	27 ± 6	24 ± 4	21 ± 4	20 ± 5
Ibuprofen	100	37 ± 6	37 ± 5	32 ± 4	28 ± 3	26 ± 5	26 ± 6‡
Ibuprofen	50	38 ± 5	31 ± 5	23 ± 6	24 ± 7	20 ± 6	18 ± 5
Aminopyrine	20	37 ± 4	36 ± 4	35 ± 5	31 ± 6	28 ± 5	26 ± 5‡
Aminopyrine	5	40 ± 5	29 ± 6	25 ± 4	23 ± 3	17 ± 3	14 ± 4
Flufenamic acid	10	41 ± 6	39 ± 6	33 ± 5	31 ± 6	29 ± 4	27 ± 5‡
Flufenamic acid	2	40 ± 7	30 ± 5	31 ± 6	27 ± 4	21 ± 5	20 ± 4
Meclofenamic acid	10	42 ± 6	40 ± 7	37 ± 5	32 ± 5	29 ± 6	28 ± 5‡
Meclofenamic acid	1	40 ± 7	36 ± 6	34 ± 5	31 ± 5	27 ± 4	25 ± 4‡
Salicylic acid	100	39 ± 6	36 ± 6	30 ± 7	29 ± 4	28 ± 5	25 ± 5‡
Salicylic acid	50	38 ± 5	32 ± 5	30 ± 5	30 ± 3	26 ± 4	24 ± 4
Phenylacetic acid	20	36 ± 5	29 ± 4	27 ± 6	21 ± 5	19 ± 6	14 ± 5
Hydrocortisone	50	39 ± 7	31 ± 6	28 ± 6	23 ± 5	20 ± 5	18 ± 4
Chloroquine	10	36 ± 6	25 ± 6	22 ± 5	20 ± 5	15 ± 4	10 ± 3
Benzindamine	10	38 ± 7	27 ± 7	24 ± 5	20 ± 4	14 ± 5	12 ± 4
Isoprenaline	10	39 ± 8	30 ± 4	28 ± 5	26 ± 3	22 ± 4	20 ± 3
Aminophylline	50	39 ± 6	29 ± 5	28 ± 6	27 ± 3	21 ± 5	19 ± 5

* Mean of at least 30 observations ± s.e. mean.
‡ A significant difference exists between the value marked † and values marked ‡ (Student's t test, $P < 0.05$).

Table 3 Membrane potential of endothelial and mesothelial cells from various tissues of the guinea-pig

Tissue	*Temperature (°C)*	*Concentration of $CaCl_2$ (mM)*	*Concentration of histamine (μg/ml)*	*Membrane potential† (mV negative)*
Thoracic aorta	37	0*	0	41 ± 7
Thoracic aorta	37	1	0	37 ± 4
Thoracic aorta	45	1	0	28 ± 5
Thoracic aorta	37	1	100	22 ± 4
Inferior vena cava	37	0	0	39 ± 6
Inferior vena cava	37	1	0	35 ± 5
Inferior vena cava	45	1	0	23 ± 4
Inferior vena cava	37	1	100	22 ± 6
Hepatic portal vein	37	0	0	43 ± 5
Hepatic portal vein	37	1	0	36 ± 7
Hepatic portal vein	45	1	0	25 ± 4
Hepatic portal vein	37	1	100	20 ± 3
Jejunal mesentery	37	0	0	30 ± 4
Jejunal mesentery	37	1	0	28 ± 4
Jejunal mesentery	45	1	0	16 ± 5
Jejunal mesentery	37	1	100	14 ± 3

† After exposure to the stated conditions for 1 hour. The mean of at least 30 observations ± s.e. mean. * Zero signifies that no calcium was added and the superfusate was made up with 0.1 mM EGTA.

small blood vessels in the tongue and foot web of the frog which were a little greater than those found in the present work. Differences between experimental conditions may account in part for the discrepancy. In addition, differences probably exist between the membrane potentials of endothelial cells in blood vessels of different size and location and particularly from different species. Action potentials were not observed by Funaki, or in the present experiments.

Aortic endothelial cells were depolarized by several monovalent metal cations in the present work. The presence or absence of calcium made little difference to the depolarizing effect of potassium. This suggests that, in contrast to the situation in certain other tissues, there is little interaction between these two ions in the endothelial cell membrane. The depolarizing action of potassium has been reported by other workers in a variety of tissues (Adrian, 1956; Dean & Matthews, 1970; Kanno, 1972; Okado, Ogawa, Aoki & Izutsu, 1973). The reduction in membrane potential for a tenfold increase in potassium concentration was 21 mV in the absence of calcium and 20 mV in the presence of calcium 1 mM. These are lower values than have been reported in certain other tissues and indicate that the membrane potential of endothelial cells is dependent upon the trans-membrane concentration gradient of other ions in addition to potassium.

Electrically excitable cells usually depolarize in the absence of extracellular calcium (Frank & Inoue, 1973). Smooth muscle cells appear to retain their membrane potential in the absence of calcium provided that magnesium ions are present (Bülbring & Tomita, 1970). Certain non-excitable cells actually show a hyperpolarization when extracellular calcium is removed (Kanno, 1972). This latter study, however, was conducted in the continued presence of magnesium. In the present experiments it was found that removal of calcium from the bathing fluid did not cause depolarization, whether or not magnesium ions were present. Moreover, the addition of calcium to the bathing fluid in supra-physiological amount actually caused depolarization. Most significantly of all, a concentration of calcium normally present in the blood plasma caused enhancement of the depolarizing effects of histamine and heating to 45° C.

The ability of the trivalent cations of lanthanum, aluminium and iron to antagonize the action of calcium was not totally unexpected since a similar antagonism has been reported for lanthanum by van Breemen, Fabrinas, Casteels, Gerba, Wuytack & Deth (1973). A comparison of the calcium-antagonizing actions of lanthanum, aluminium and iron does not appear to have been made previously. Nevertheless, some of the published pharmacological actions of aluminium are most readily explained in terms of inhibition of the effects of endogenous calcium (Hava & Hurwitz, 1973).

Firm conclusions regarding the possible relationships between the membrane potential of endothelial cells and the leakage of plasma through the endothelium of small blood vessels during inflammation are obviously not yet possible. Four points, however, are worthy of note. Firstly, exposure of mammalian tissues to histamine or to a temperature of 45° C both cause inflammation (Starr & West, 1967). Secondly, the present findings indicate that either histamine or heating to 45° C can depolarize endothelial cells, the effects of both being enhanced by a concentration of calcium existing normally in the blood plasma. Thirdly, indomethacin and members of a group of pharmacologically related substances reduce the binding of calcium to endothelial cell membranes (see Introductory section). Fourthly, these same drugs have been shown in the present experiments to inhibit the ability of calcium to augment the histamine-induced and heat-induced depolarization of endothelial cells. One can only speculate as to how these four separate series of observations are connected. Perhaps, by binding to the endothelial cell membrane, the calcium ions normally present in the blood plasma facilitate inflammatory responses to histamine or heat. In which case, perhaps it is by reducing the binding of calcium to endothelial cell membranes that drugs such as indomethacin exert their anti-inflammatory effect

References

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

BIANCHI, C.P. (1968). *Cell Calcium*, p. 53. London: Butterworths.

BULBRING, E. & TOMITA, T. (1970). Calcium and the action potential in smooth muscle. In: *Calcium and Cellular Function*. ed. Cuthbert, A.W., pp. 249-260. London: Macmillan.

DEAN, P.M. & MATTHEWS, E.K. (1970). Electrical activity in pancreatic islet cells: effect of ions. *J. Physiol., Lond.*, **210**, 265-275.

DURANT, G.J., SMITH, G.M., SPICKETT, R.G.W. &

SZARVASI, E. (1965). Nonsteroidal anti-inflammatory agents. Some arylacetic acids. *J. med. Chem.*, 8, 598-603.

FRANK, G.B. & INOUE, F. (1973). Effects of reducing the external calcium concentration on the resting potential of frog's skeletal muscle fibers. *Jap. J. Physiol.*, **23**, 183-197.

FUNAKI, S. (1961). Spontaneous spike discharges of vascular smooth muscle. *Nature, Lond.*, **191**, 1102-1103.

HAVA, M. & HURWITZ, A. (1973). The relaxing effect of aluminium and lanthanum on rat and human gastric smooth muscle *in vitro*. *Eur. J. Pharmac.*, **22** 156-161.

KANNO, T. (1972). Calcium-dependent amylase release and electrophysiological measurements in cells of the pancreas. *J. Physiol., Lond.*, **226** 353-371.

NORTHOVER, B.J. (1964). The action of anti-inflammatory compounds in mice with peritonitis. *J. Path. Bact.*, 87, 395-404.

NORTHOVER, B.J. (1972). The effects of indomethacin on calcium, sodium, potassium and magnesium fluxes in various tissues of the guinea-pig. *Br. J. Pharmac.*, **45**, 651-659.

NORTHOVER, B.J. (1973). Effect of anti-inflammatory drugs on the binding of calcium to cellular membranes in various human and guinea-pig tissues. *Br. J. Pharmac.*, **48**, 496-504.

OKADO, Y., OGAWA, M., AOKI, N. & IZUTSU, K. (1973). The effect of K^+ on the membrane potential of HeLa cells. *Biochim. Biophys. Acta*, **292**, 116-126.

STARR, M.S. & WEST, G.B. (1967). Bradykinin and oedema formation in heated paws of rats. *Br. J. Pharmac. Chemother.*, **31**, 178-187.

VAN BREEMEN, C., FARINAS, B.R., CASTEELS, R., GERBA, P., WUYTACK, F. & DETH, R. (1973). Factors controlling cytoplasmic Ca^{2+} concentration. *Phil. Trans. R. Soc. Lond. B.*, **265**, 57-71.

(Revised May 30, 1974)