

The inhibitory effect of aspirin on lymphatic contractility

J.M. Allen, E.P. Burke, M.G. Johnston* & N.G. McHale**

School of Life Sciences, Ulster Polytechnic, Newtownabbey, County Antrim, BT37 0QB, Northern Ireland, Division of Experimental Pathology*, Department of Pathology, University of Toronto, Medical Sciences Building, Toronto, Ontario, Canada and Department of Physiology**, Queen's University, Belfast, Northern Ireland.

- 1 Spontaneous contractions and those elicited by two different methods of electrical stimulation were studied in isolated segments of bovine mesenteric lymphatic vessels.
- 2 The effect of aspirin (a cyclo-oxygenase inhibitor) on spontaneous and evoked contractions of isolated lymphatic vessels was investigated.
- 3 Aspirin at doses of 10^{-6} M or greater depressed both spontaneous and action potential-dependent evoked contractions, but failed to inhibit contractions evoked by high current field stimulation. These latter contractions were rapidly depressed by the application of D600.
- 4 When aspirin was applied for five minute periods, inhibition never occurred within the period of drug administration but was delayed, with maximum inhibition occurring approximately 10 min after washout of the drug.
- 5 It is concluded that the inhibitory action of aspirin is unlikely to be a non-specific depression of the contractile mechanism, but rather a reduction in excitability probably as a result of cyclo-oxygenase inhibition.

Introduction

Segments of bovine mesenteric lymphatic vessels show regular spontaneous contractions *in vitro* and are capable of propelling fluid by this intrinsic activity (McHale & Roddie, 1976). Each contraction is preceded and initiated by a single action potential and relaxation is complete before a second contraction occurs (Allen *et al.*, 1983). This regular contractile activity is not neurogenic, since tetrodotoxin has no obvious effect, and much investigation has been directed towards determining those factors that may serve to initiate and control this activity. For example, it has been shown that both rate and force of contraction may be modified by distension of the vessel wall, administration of exogenous noradrenaline and stimulation of the intramural nerves which these vessels contain (Mawhinney & Roddie, 1973; McHale & Roddie, 1976; McHale *et al.*, 1980; Allen *et al.*, 1983).

More recently, attention has focused on the possibility that arachidonate metabolites (produced by the lymphatic vessels or entering in the lymph draining the tissue) may regulate lymph flow through direct effects on lymphatic contractility. Nanomolar con-

centrations of prostaglandin endoperoxide PGH_2 , a stable PGH_2 analogue, and the leukotrienes B_4 , C_4 and D_4 elicited rhythmical contractions and increased tone in quiescent vessel segments (Johnston & Gordon, 1981; Johnston *et al.*, 1983). Further, when vessels showed inherent spontaneous contractility this activity could be blocked by aspirin and indomethacin (cyclo-oxygenase inhibitors), BW755C (a cyclo-oxygenase and lipoxygenase inhibitor) and FPL55712 (a leukotriene antagonist) (Johnston & Feuer, 1983). It was suggested that the latter agents exerted their inhibitory effects indirectly via blockade of endogenous prostaglandin production, or antagonism of one or more of the arachidonate products, although no direct evidence was available for this.

The purpose of this investigation was to examine the effect of aspirin on spontaneously contracting lymphatics and on those driven to contract by electrical stimulation. Electrical field stimulation of lymphatic segments may evoke two different types of response depending on the duration and strength of the applied field. Stimulation with square-wave

pulses of relatively short duration (< 100 ms) and low electrode voltage (< 10 V) elicits action potential-dependent responses which are very similar in amplitude and duration to the spontaneously generated response, being dependent on a polarized membrane and requiring conduction of excitation along the vessel segment. In contrast, when stimulated with pulses of relatively long duration (> 2 s) and high electrode voltage (> 50 V) contractions evoked are of longer duration than spontaneous ones and their amplitude is dependent on the voltage and duration of the applied pulse. Indeed, contractions of the latter type may be evoked even after depolarization in high K^+ Krebs solution (Sperelakis, 1975; Allen *et al.*, 1981). Since this latter method bypasses at least part of the normal excitation/contraction coupling process, this method of stimulation may be used to examine the effect of agents on contraction subsequent to the action potential.

Methods

Sections of mesenteric lymphatics, approximately 3 cm in length and 2 mm in diameter, were dissected

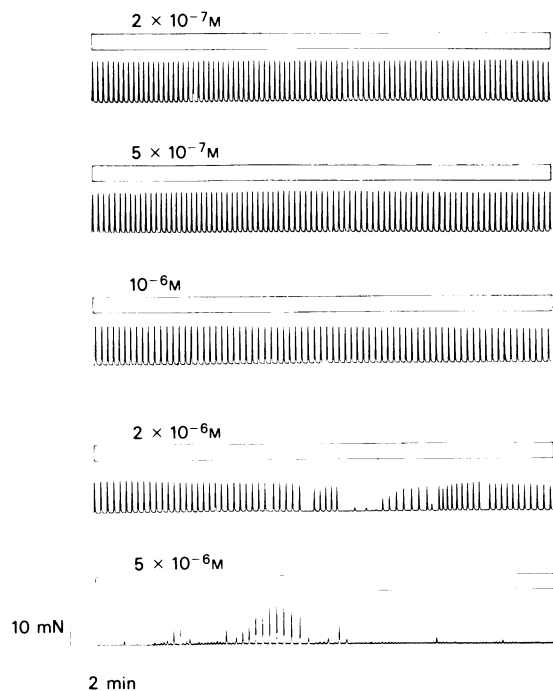


Figure 1 Effect of aspirin at five different concentrations on a spontaneously active isolated lymphatic vessel. Aspirin concentration is shown above each record. Records are continuous.

free from the mesenteries of freshly slaughtered cattle and mounted in a water-jacketed organ bath maintained at 35°C . The lower end of the vessel was fixed and the upper end attached to an isometric force transducer (Statham UC3) for measurement of longitudinal tension. The transducer output was amplified and displayed on a Brush 2200 chart recorder. The organ bath volume was 3 ml and was perfused with pre-heated Krebs solution at a rate of 6 ml min^{-1} . Each preparation was initially placed under 2 mN tension and left to equilibrate for a period of approximately 45 min. After this time vessels were normally spontaneously active and were either allowed to continue to contract spontaneously or were driven to contract at a fixed rate by electrical stimulation. For the latter purpose platinum ring electrodes were positioned at the top and bottom of the organ bath and connected to a pulse generator (Grass S88).

Solutions and drugs used were as follows: Krebs solution containing (mM) NaCl 118, KCl 4.7, NaHCO_3 25, NaH_2PO_4 1.25, MgCl_2 1.2, CaCl_2 2.5, and glucose 5.55. The solution was gassed with 5% CO_2 in O_2 and had a pH of 7.4 at 35°C . Aspirin (acetylsalicylic acid, Sigma) and D600 hydrochloride (methoxyverapamil, Knoll A.G.) were made up to final concentrations in Krebs solution when required.

Results

Spontaneous activity

The effect on spontaneously active vessels of increasing aspirin concentrations from 10^{-8} to 10^{-5} M was investigated. The dose was increased in a cumulative manner with each concentration being present for a 30 min period. At less than 10^{-6} M aspirin had little or no obvious effect on either rate or force of contraction, although higher concentrations reduced contraction force and progressively disrupted spontaneous contraction frequency, to the point of total abolition at the highest dose.

Figure 1 illustrates one experiment of the type described above. Aspirin concentrations of 2×10^{-7} and 5×10^{-7} M had no obvious effect on vessel activity. At 10^{-6} M, frequency and force of contraction were slightly reduced during the period of administration. With 2×10^{-6} M, significant disruption of contraction frequency occurred and activity transiently stopped following introduction of the highest concentration shown. Nevertheless, on addition of aspirin at 5×10^{-6} M small oscillations of the tension record are evident and these occasionally developed into complete contraction of the vessel. At 10^{-5} M aspirin, all mechanical activity ceased and 1 h after washout of the drug the vessel had not returned to normal activity (not shown). The effect of aspirin on

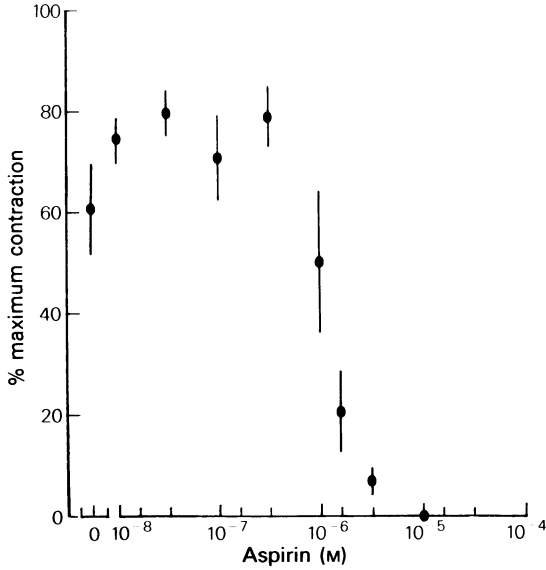


Figure 2 Summary of seven experiments on the effect of aspirin on contraction amplitude when lymphatic vessels were spontaneously active. Amplitude of contraction is plotted as a percentage of the maximum spontaneous contraction occurring in each experiment. Vertical lines represent s.e.mean. For further details see text.

the amplitude of contraction for a series of experiments is summarized in Figure 2. The average height of contraction during the final 15 min of each aspirin addition was expressed as percentage of the maximum spontaneous contraction for each vessel. Means of these percentage values from seven experiments were used to produce Figure 2. It would appear from this figure that aspirin at concentrations $> 5 \times 10^{-7} M$ reduces lymphatic contractility in a

dose-dependent fashion. However, aspirin exerts this effect not simply by reducing individual contraction force but also by disrupting spontaneous pacemaking so that some contractions are eliminated entirely (original record of Figure 1). Indeed, even with $10^{-5} M$ aspirin, which invariably blocked spontaneous activity, vessels were often capable of responding to electrical stimulation with contractions which were equal in amplitude to those of the control.

Evoked activity

(a) *Action potential-dependent contractions* In this series of experiments vessels were stimulated to produce approximately one contraction per min by 100 ms pulses $< 10 V$ (nominal).

In initial experiments aspirin, at concentrations between 10^{-8} and $10^{-4} M$, was added for a 5 min period to the fluid bathing the tissue and the effect on vessel contractility recorded. Approximately 25 min was allowed between successive additions. Concentrations of aspirin $< 5 \times 10^{-7} M$ were without observable effect, although higher concentrations than this reduced contractility in a dose-dependent fashion. Figure 3 illustrates the effect of aspirin, at concentrations of 5×10^{-6} and $10^{-5} M$, on one preparation. Note that the inhibitory effect was dose-dependent and delayed in time, with maximum inhibition occurring about 10 min after washout of the drug. This delay in onset of inhibition was typical of all the vessels studied. Note further that the inhibition following $10^{-5} M$ aspirin was not just a simple and progressive reduction in contraction force but was punctuated by intermittent failure of any significant response to stimulation. This reduction in vessel excitability was characteristic of the effect of aspirin in five of the seven experiments of this type.

To examine the effect of aspirin under steady-state conditions further experiments were carried out in

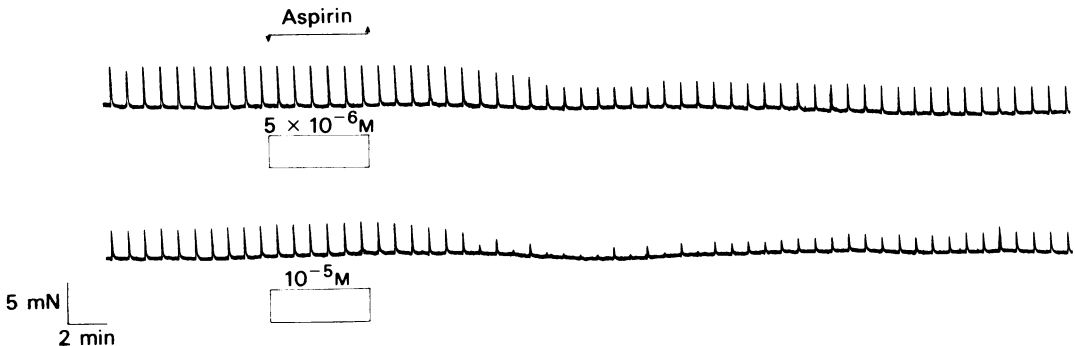


Figure 3 Effect of aspirin at two concentrations on the contractility of one lymphatic vessel when contractions were evoked by electrical stimulation (100 ms pulses, 10 V (nominal)). Aspirin was present for a 5 min period as indicated above the records. Concentration is shown below each record. Upper and lower records are continuous.

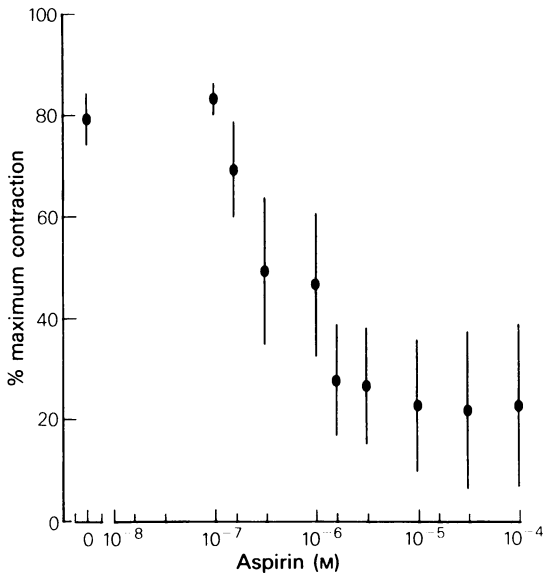


Figure 4 Summary of seven experiments on the effect of aspirin on contraction amplitude when lymphatic vessels were electrically stimulated by 100 ms pulses of < 10 V (nominal). The contraction amplitude is expressed as a percentage of the maximum contraction occurring in each experiment (as in Figure 2). Vertical lines represent s.e.mean.

which increasing aspirin concentrations were added cumulatively to the fluid perfusing the organ bath; each concentration being present for a 30 min period. Figure 4 summarizes seven such experiments. In five of the experiments aspirin reduced contractility in a dose-dependent manner resulting in a complete inhibition of contraction at concentrations between 10^{-6} and 5×10^{-5} M. However, in the remaining two experiments an initial reduction in contraction amplitude was followed by recovery as the aspirin concentration was increased.

(b) *Action potential independent contractions* In these experiments, vessels were stimulated to produce approximately one contraction per min by single 2 s pulses at 50 V (nominal). When vessels were stimulated in this way, aspirin at concentrations up to and including 10^{-4} M had no obvious effect on vessel contractility (Figure 5). Note that at concentrations which were shown to reduce contractility under the conditions of the previous series of experiments, aspirin failed to reduce contraction force (cf Figure 4). Indeed, aspirin at 10^{-4} M was ineffective even if perfused for a 30 min period of time, although contractions could be quite rapidly abolished by a lower dose of the calcium antagonist D600 (10^{-5} M, Figure 6).

Discussion

The fact that various arachidonic acid metabolites have a potent effect on lymphatic smooth muscle contractility (Johnston & Gordon, 1981; Johnston *et al.*, 1983) and that lymph draining inflammatory sites contains large quantities of these substances (Jonsson *et al.*, 1979; Johnston *et al.*, 1979; 1980; Demling *et al.*, 1981) makes it likely that they are involved in the regulation of lymphatic contractility and thus lymph flow. The observations that inhibitors of arachidonate metabolism are capable of suppressing lymphatic vessel contractions (Johnston & Feuer, 1983) has led to the hypothesis that arachidonate metabolites within the lymphatic vessel may provide some control of the contractile responses. Although the nature of the latter mechanism is unclear it is possible that they are involved in providing a background excitability which would facilitate the responses of the vessel to stretch, circulating catecholamines and nerve stimulation (McHale & Roddie, 1976; 1983; McHale *et al.*, 1980). It is to be expected therefore that the cyclo-oxygenase inhibitor, aspirin, would reduce this

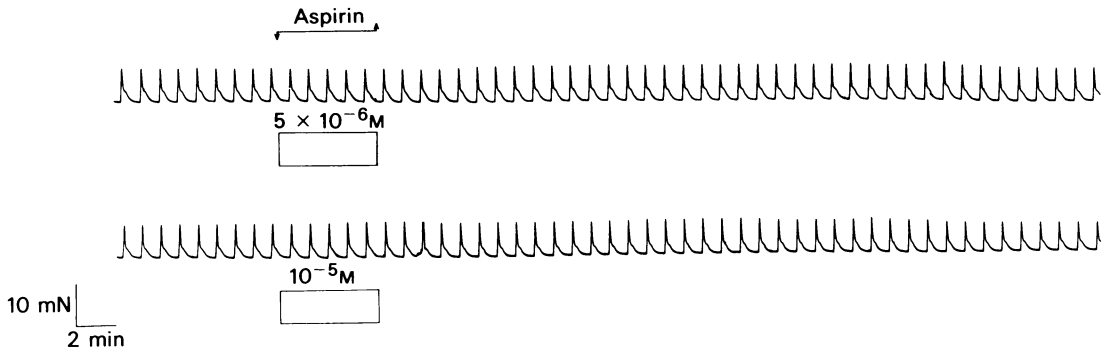


Figure 5 Effect of aspirin at two concentrations on the contractility of an isolated lymphatic vessel when contractions were evoked by field pulses of 2 s duration at 50 V (nominal). Aspirin was present for a 5 min period as indicated above the records. Concentration is shown below each record. Records are continuous.

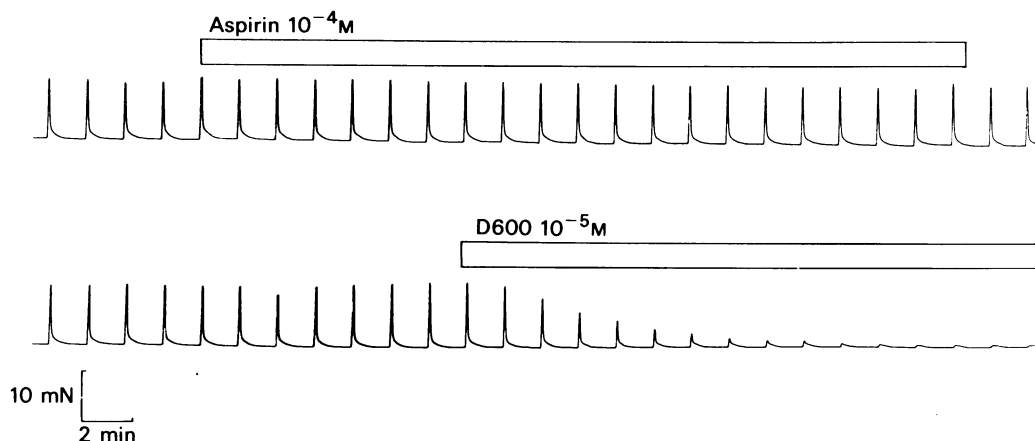


Figure 6 Upper trace shows that when contractions were evoked by field stimulation with 2 s pulses at 50 V (nominal) even prolonged perfusion with aspirin (10^{-4} M) failed to reduce contraction force. However, contraction was rapidly inhibited by D600 (10^{-5} M) (lower trace). Aspirin and D600 were present for the periods indicated above the records. There is a 10 min gap between the upper and lower records.

level of excitability. The results of this study amply confirm these expectations. That the action is to disrupt or depress propagated electrical activity is confirmed by the observations that spontaneous contractions and action potential-dependent evoked contractions are depressed when doses of 10^{-6} M or greater are used. However, inhibitors of arachidonate metabolism have also been shown to reduce the effects of several agonists (Johnston & Feuer, 1983) raising the possibility that they may have some non-specific depressant activity not related to their ability to inhibit arachidonate metabolism. The observations reported here make it unlikely that the response to aspirin is a non-specific depression of contractility since contractions evoked by high current field stimulation (which is known to bypass the action potential mechanism) are unaffected by doses as high as 10^{-4} M. In contrast, the latter type of contractions are depressed by the calcium antagonist D600 or by β -adrenoceptor agonists (Allen *et al.*, 1983) which are known to have a direct inhibitory effect.

However, these results do not confirm that aspirin depresses lymph vessel excitability by inhibiting cyclo-oxygenase. Nevertheless they do provide some indication that this is at least part of the mechanism of action. When, for example, the drug was applied for five min periods and then the vessels were returned to normal Krebs solution, inhibition never occurred within the period of drug administration even though it was during this time that a maximum concentration of the drug was achieved. Depression of contraction did not occur until ten min after drug washout, a delay that could be accounted for in terms of a metabolic action since those drugs which are known to have a direct inhibitory effect (such as isoprenaline) normally exert this during the first few minutes of drug addition (McHale & Roddie, 1983; Allen *et al.*, 1983).

The authors' thanks are due to Mr Colin Graham for excellent technical assistance and to Knoll, A.G. for providing D600.

References

- ALLEN, J.M., MCHALE, N.G. & ROONEY, B.M. (1981). Direct stimulation of lymphatic smooth muscle. *Irish J. med. Sci.*, **150**, 96.
- ALLEN, J.M., MCHALE, N.G. & ROONEY, B.M. (1983). Effect of norepinephrine on contractility of isolated mesenteric lymphatics. *Am. J. Physiol.*, **244**, H479-H486.
- DEMLING, R.H., SMITH, M., GUNTHER, R., FLYNN, J.T. & GEE, M.H. (1981). Pulmonary injury and prostaglandin production during endotoxemia in conscious sheep. *Am. J. Physiol.*, **240**, H348-H353.
- JOHNSTON, M.G. & FEUER, C. (1983). Suppression of lymphatic vessel contractility with inhibitors of arachidonic acid metabolism. *J. Pharm. exp. Ther.*, (in press).
- JOHNSTON, M.G. & GORDON, J.L. (1981). Regulation of lymphatic contractility by arachidonate metabolites. *Nature*, **293**, 294-297.
- JOHNSTON, M.G., HAY, J.B. & MOVAT, H.Z. (1979). Kinetics of prostaglandin production in various inflammatory lesions, measured in draining lymph. *Am. J. Pathol.*, **95**, 225-238.
- JOHNSTON, M.G., HAY, J.B. & MOVAT, H.Z. (1980). The

- distribution of prostaglandins in afferent and efferent lymph from inflammatory sites. *Am. J. Pathol.*, **99**, 695–714.
- JOHNSTON, M.G., KANALEC, A. & GORDON, J.L. (1983). Effects of arachidonic acid and its cyclo-oxygenase and lipoxygenase products on lymphatic vessel contractility in vitro. *Prostaglandins*, **25**, 85–98.
- JONSSON, C.E., SHIMIZU, Y., FREDHOLM, B.B., GRANSTRÖM, E. & OLIW, E. (1979). Efflux of cyclic-AMP, prostaglandin E₂ and F_{2α} and thromboxane B₂ in leg lymph of rabbits after scalding injury. *Acta physiol. scand.*, **107**, 377–384.
- MAWHINNEY, J.D. & RODDIE, I.C. (1973). Spontaneous activity in isolated bovine mesenteric lymphatics. *J. Physiol.*, **229**, 339–348.
- McHALE, N.G. & RODDIE, I.C. (1976). The effect of transmural pressure on pumping activity in isolated bovine lymphatic vessels. *J. Physiol.*, **261**, 255–269.
- McHALE, N.G. & RODDIE, I.C. (1983). The effect of intravenous adrenaline and noradrenaline infusion on peripheral lymph flow in the sheep. *J. Physiol.*, **341**, 517–526.
- McHALE, N.G., RODDIE, I.C. & THORNBURY, K.D. (1980). Nervous modulation of spontaneous contractions in bovine mesenteric lymphatics. *J. Physiol.*, **309**, 461–472.
- SPERELAKIS, N. (1975). Electrical stimulations of smooth muscle: Field stimulation. In *Methods in Pharmacology*, Vol. 3. *Smooth muscle*, ed. Daniel, E.E. & Paton, D.M., pp. 321–337. New York: Plenum Press.

(Received November 21, 1983.

Revised January 30, 1984.)