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THE EFFECTS OF ISOPRENALINE AND BRADYKININ ON CAPILLARY FILTRATION IN THE CAT SMALL INTESTINE

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1 Lymph flow, and both lymph and plasma protein concentrations were measured in isolated, blood-perfused loops of cat ileum.

2 Permeability-surface area (PS) products and the osmotic reflection coefficient (σ) of the intestinal capillaries were calculated.

3 Isoprenaline (one dose) or bradykinin (two different doses) was infused into the superior mesenteric artery.

4 Isoprenaline (blood concentration about 50 ng/ml) did not affect PS or σ.

5 Bradykinin (about 36 ng/ml) increased PS but as σ was unaltered, this was primarily due to an increased capillary surface area.

6 Bradykinin (about 680 ng/ml) increased PS and as σ was reduced, there was an increased capillary permeability.

7 Reasons for the lack of effect of isoprenaline at concentrations which increase capillary filtration coefficient are discussed.

8 These data show that this technique separates drug effects on capillary surface area from effects on capillary permeability.

Introduction

It is established that both isoprenaline and bradykinin cause an increase in blood flow and capillary filtration coefficient (CFC) in the small intestine of the anaesthetized cat, effects which are apparent on both intra-arterial (Folkow, Lundgren, & Wallentin, 1963; Fasth, 1973; Fasth & Hulten, 1973) and intravenous (Richardson, 1973; 1974) administration. Increases in CFC are interpreted as resulting from an increased exchange vessel surface area, or increased microvascular permeability, or both (Folkow *et al.*, 1963; Mellander & Johansson, 1968; Folkow & Mellander, 1970).

The distinction between increased surface area and increased vascular permeability as a cause of drug-induced increases in CFC in the intestine relies upon indirect evidence, and the results of studies in other tissues (Fasth & Hulten, 1973; Richardson, 1974; 1976). This approach suggests that the increase in CFC due to isoprenaline is primarily due to an increase in exchange vessel area (precapillary 'sphincter' dilation), whilst that due to bradykinin is primarily due to increased permeability. Although it is well-established that bradykinin can increase microvascular permeability (Svensjo, Persson & Rutili, 1977), it has not been established that this occurs in the absence of surface area changes, or that it occurs at bradykinin concentrations which evoke changes in CFC. Similarly, it has not been established that isoprenaline increases CFC solely by increasing exchange vessel surface area.

Pathophysiologically and therapeutically, the distinction between an effect on vascular surface area and permeability may be of importance. The present study was designed to investigate the effects of intra-arterial infusions of both isoprenaline and bradykinin on vascular surface area and permeability in the cat small intestine, by examining the effects of these drugs on intestinal lymph flow and the ratio between the concentration of protein in intestinal lymph, and in plasma.

Methods

Experiments were performed on a total of 24 cats which had been deprived of food for 24 h but allowed

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access to water *ad libitum* prior to an intramuscular injection of 65 to 80 mg/kg ketamine hydrochloride (Ketaject, Bristol Laboratories) to induce anaesthesia. Pentobarbitone sodium (Butler) was injected into a cannulated femoral vein as necessary during the experiments to maintain a constant level of anaesthesia.

The preparations were similar to those described previously (Granger, Valleau, Parker, Lane & Taylor, 1978): following cannulation of the trachea and a femoral artery (for measurement of systemic arterial pressure), a midline laparotomy was performed and the duodenum, jejunum, spleen, omenta, and part of the ileum extirpated. The periarterial sympathetic nerves to the remaining loop (about 50 g) of ileum were divided, the animals heparinized (5 mg/kg, i.v.), and the superior mesenteric artery cannulated and perfused at essentially constant pressure from a cannulated common carotid artery. The superior mesenteric vein was cannulated, and the outlet from the cannula arranged so that it could be raised or lowered, thereby controlling the superior mesenteric venous pressure. The external vascular circuit was primed with heparinized whole blood obtained from a freshly-killed donor cat.

Intestinal lymph flow was measured in a large lymphatic vessel which was cannulated between the intestinal pedicle and the superior mesenteric lymph node.

Measurements

Blood flow and pressures Systemic arterial pressure (BP) was measured from a cannulated femoral artery, by means of a Statham P23Ac transducer. Superior mesenteric venous pressure (P_V) was measured from a 't'-piece in the cannula draining the superior mesenteric vein, positioned close to the point of cannulation of the vein, with a Statham P23Bc transducer. Zero reference venous pressure was taken at the level of the intestinal loop; pressure transducers were calibrated with mercury or saline manometers. Superior mesenteric venous blood flow was measured with a flow probe in the cannula leading from the superior mesenteric vein and a Carolina Medical square-wave electromagnetic flowmeter; this system was calibrated *in situ* by timed collections of whole blood.

These variables were recorded on a Grass 7D polygraph, mean pressures and blood flow being obtained by passing the signals through averaging circuits with time constants of about 2 s.

Lymph flow Lymph flow was measured by timing the movement of lymph along graduated micropipettes (50 to 200 µl or 1 ml capacity) positioned horizontally at the level of the intestinal loop and pedicle.

Protein concentrations The concentration of protein in the lymph samples (C_L) was measured with an American Optical refractometer. Concomitantly, a sample of systemic arterial blood was taken, centrifuged, and the plasma protein concentration (C_P) determined in the same way.

Calculations

The loops of gut were weighed *post mortem*; blood flow and lymph flow were expressed per 100 g gut weight. Intestinal vascular resistance was calculated as (systemic arterial − superior mesenteric venous pressure)/(intestinal blood flow) and expressed as mmHg ml^{-1} min 100 g.

The ratio of lymph to plasma protein concentration (C_L/C_P) was calculated from lymph and plasma samples obtained concomitantly.

Drugs

Isoprenaline hydrochloride (Isoprel, Winthrop) and bradykinin triacetate (Sigma) were dissolved in, diluted from ampoules with Tyrode solution, and infused into the cannula leading to the superior mesenteric artery in volumes not exceeding 0.05 ml/min by means of a Harvard slow-infusion pump. Fresh solutions were prepared for each experiment; doses are expressed as weights of the salts. When intestinal blood flow varied during the course of the experiments (e.g. on elevation of the venous pressure), the infusion rates were adjusted to maintain a calculated superior mesenteric arterial blood concentration within about 10% of the initial calculated drug concentration.

Presentation of results

The animals were divided into 4 groups: group i ($n = 9$) control group receiving no drugs; group ii ($n = 5$) isoprenaline; group iii ($n = 3$) low dose of bradykinin, and group iv ($n = 7$) high dose of bradykinin. In each experiment, the cardiovascular variables, lymph flows (J_L) and lymph and plasma protein concentrations were determined under steady-state conditions at venous pressures of 0, 10, 20 and 30 mmHg. The values for (C_L/C_P) were calculated at the various lymph flows induced by increasing the intestinal venous pressure. At high lymph flows, the deviation of (C_L/C_P) from unity is principally determined by the restriction of protein which is offered by the capillary membrane. The index [1-(C_L/C_P)] is therefore a measurement of the degree of restriction of protein movement offered by the capillary pores and, at high lymph flows, gives a quantitative measurement of the capillary permeability. The value for [1-(C_L/C_P)] at high lymph flows (Figure 1) is the

osmotic reflection coefficient for the plasma proteins (σ), as shown by Brace, Granger & Taylor (1977). The osmotic reflection coefficient is an extremely sensitive measure of capillary permeability which is independent of capillary surface area. Figure 1 illustrates the effect of σ on the relationship between C_L/C_P and lymph flow. As lymph flow increases C_L/C_P decreases rapidly and then becomes relatively constant as C_L/C_P approaches a minimal value. Previous studies indicate that using $[1-(C_L/C_P)]$ at the level where (C_L/C_P) no longer changes with lymph flow provides an estimate of σ (Taylor, Granger & Brace, 1977; Brace, *et al.*, 1977). In the present study capillary permeability (σ) was assessed using this approach.

In order to determine the effect of isoprenaline and bradykinin on capillary surface area we estimated the permeability surface area product for plasma proteins by the equation (q.v. Renkin, Joyner, Sloop, & Watson, 1977):

$$PS = \frac{(J_L \times C_L) - J_L(C_P(1-\sigma))}{C_P - C_L}.$$

Permeability-surface area products were estimated from values only at intestinal venous pressure of 0 mmHg in order to eliminate the influence of myogenic reductions in capillary surface area which would occur at higher venous pressures (Johnson & Hanson, 1966).

Statistics

Variables are expressed as means ± s.e. means; the significance of differences between sets of data was assessed by paired or unpaired Student's *t* tests, as appropriate.

Results

Control values

Under control conditions in 24 animals, the systemic arterial mean pressure was 100 ± 3 mmHg, and the superior mesenteric venous blood flow 25.4 ± 2.4 ml min^{-1} 100 g^{-1} at a venous pressure of zero, giving a calculated ileal vascular resistance of 4.60 ± 0.77 mmHg ml^{-1} min 100 g. The lymph flow was 0.030 ± 0.005 ml min^{-1} 100 g^{-1}, the ileal loops weighing 43.7 ± 2.6 g. The calculated values for the ratios between plasma and lymph protein concentration (C_L/C_P) and for the permeability-surface area product (PS) fell within the range of values reported previously from this laboratory for identical preparations (Granger, Brace, Parker & Taylor, 1979; Taylor *et al.*, 1977) as shown in Table 1.

Calculation of the osmotic reflection coefficient (σ)

In a series of 9 experiments, the superior mesenteric venous pressure was elevated in steps of 10 mmHg from 0 to 30 mmHg and once a steady-state lymph flow had been attained at each venous pressure, duplicate determinations of the lymph and plasma protein concentrations were made. The ratio (C_L/C_P) of these values was calculated and plotted against the lymph flow, as shown in Figure 1 (open circles). From Figure 1, it is apparent that the minimal value for (C_L/C_P) is about 0.08, and consequently, the value for σ, calculated as $[1-(C_L/C_P)]$ is 0.92.

Effects of isoprenaline

In a separate series of 5 experiments, lymph flow, lymph and plasma protein concentrations were deter-

Table 1 Effects of isoprenaline and bradykinin on intestinal lymph flow, capillary surface area and permeability

	Drug and arterial blood concentration (ng/ml)	*Lymph flow ($ml\ min^{-1}\ 100g^{-1}$)*	C_L/C_P	*PS ($ml\ min^{-1}100g^{-1}$)*	σ
A	Control	0.056 ± 0.017	0.63 ± 0.06	0.119 ± 0.084	0.92
	Isoprenaline (47 ± 17)	0.049 ± 0.019	0.60 ± 0.05	0.100 ± 0.040	0.92
B	Control	0.012 ± 0.004	0.50 ± 0.06	0.013 ± 0.007	0.92
	Bradykinin (36 ± 8)	0.056 ± 0.022**	0.61 ± 0.05**	0.089 ± 0.020**	0.92
C	Control	0.023 ± 0.006	0.48 ± 0.02	0.019 ± 0.005	0.92
	Bradykinin (683 ± 62)	0.331 ± 0.075*	0.74 ± 0.02**	0.560 ± 0.150*	0.65

C_L = lymph protein concentration, C_P = plasma protein concentration, PS = permeability-surface area, σ = osmotic reflection coefficient for plasma proteins.
All values are presented as means ± s.e. means where the number of observations is as follows: A 5; B 3; C 7. The significance of differences from control, assessed by paired *t* tests is indicated thus: $**P < 0.001$; $*P < 0.01$. Unpaired *t* tests showed no significant differences between the control values of groups A, B or C ($P > 0.1$).

mined at zero venous pressure before and then during isoprenaline infusions into the superior mesenteric artery. The infusions resulted in calculated maximum arterial blood concentrations of isoprenaline of 47 ± 17 ng/ml. These influsions caused no significant changes in total lymph flow, C_L, C_P, or the ratio (C_L/C_P), ($P > 0.20$) and calculations showed no change in the permeability-surface area product ($P > 0.80$; Table 1A).

During the maintained isoprenaline infusions, the superior mesenteric venous pressure was elevated in 10 mmHg steps to determine the osmotic reflection coefficient, and the results are shown in Figure 1 (solid circles). There was no apparent difference in the distribution of points from these experiments compared with those for the experiments performed in the absence of drugs (open circles). The minimal value for (C_L/C_P) could not therefore be differentiated from the control value, and so there was no difference in the value of σ which could be attributed to the isoprenaline infusions.

Effects of low doses of bradykinin

In a separate series of 3 experiments, following control determinations at zero venous pressure, bradykinin was infused intra-arterially, resulting in calculated maximum arterial blood concentrations of 36 ± 8 ng/ml. At zero venous pressure, these infusions resulted in statistically significant increases in total lymph flow, C_L and, since there was no significant change in plasma protein concentration ($P > 0.05$), in the ratio (C_L/C_P); the calculated value for PS was also significantly increased (Table 1B).

The superior mesenteric venous pressure was then increased in 10 mmHg steps, and the values for (C_L/C_P) at the various resultant lymph flows are shown in Figure 1 (solid squares). The data do not show any apparent difference in the minimal values for (C_L/C_P) compared with the control values in the absence of all drugs (open circles); the calculated osmotic reflection coefficient (σ) therefore cannot be distinguished from the value of 0.92 assessed for the control experiments.

Bradykinin at low arterial concentrations therefore causes an increase in the permeability-surface area product, but since the osmotic reflection coefficient remains unaltered, this cannot be ascribed to an increase in vascular permeability. The conclusion is therefore that at this dose level, bradykinin increases the functional capillary surface area of the ileum.

Effects of high doses of bradykinin

In a separate series of 7 experiments, bradykinin was infused into the superior mesenteric artery resulting

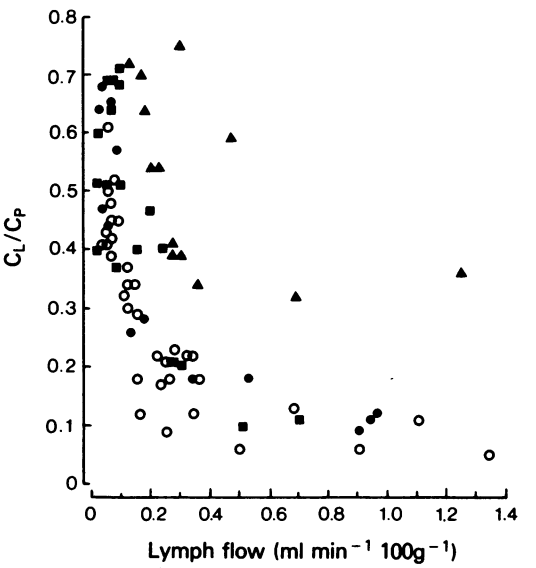

Figure 1 Relationship between lymph flow in the ileum of the cat and the ratio between lymph (C_L) and plasma (C_P) protein concentrations. The index [1-(C_L/C_P)] where (C_L/C_P) has attained a minimal value at high lymph flows induced by venous pressure elevation, gives a measurement of the osmotic reflection coefficient for plasma proteins, itself a measurement of the capillary membrane permeability. (○) Control values; (●) during isoprenaline infusions (~50 ng/ml); (■) during low infusions of bradykinin (~40 ng/ml) and (▲) during high infusions of bradykinin (~700 ng/ml).

in a calculated maximum increase in blood bradykinin concentration of 683 ± 62 ng/ml. At a venous pressure of zero, this resulted in a significant (about 15 fold) increase in lymph flow, in lymph protein concentration (about a doubling) and, since the plasma protein concentration remained unaltered ($P > 0.05$), in the ratio (C_L/C_P). The PS product was increased in all experiments, the mean increase being by a factor of nearly 30 (Table 1 C).

The venous pressure was then increased in 10 mmHg steps to 30 mmHg; the results are shown in Figure 1 (solid triangles). In contrast to the data obtained in the former experiments, these data from experiments with high doses of bradykinin revealed a marked difference in the minimal value for (C_L/C_P) obtained at high lymph flows. The minimal value of (C_L/C_P) was assessed at 0.35, giving a value of the osmotic reflection coefficient (σ) of 0.65, compared with the control of 0.92 (Table 1).

The high arterial blood concentration of bradykinin (about 0.7 μg/ml) therefore causes a substantial increase in the PS product, which includes a component due to an increase in vascular permeability since the value for σ is reduced.

Discussion

The control values for plasma and lymph protein concentrations, intestinal lymph flow and calculated PS values obtained in these experiments fell within the ranges reported previously (Taylor *et al.*, 1977; Granger *et al.*, 1979).

Isoprenaline, infused intra-arterially to produce blood concentrations of about 50 ng/ml, did not alter filtration rate, capillary surface area or permeability as judged by the criteria (lymph flow, PS product, osmotic reflection coefficient) used in the present experiments. This is perhaps a surprising finding in that similar or smaller concentrations of isoprenaline have been shown to increase volumetric estimates of the capillary filtration coefficient (CFC) in the small intestine (e.g. Folkow *et al.* (1963), about 3 ng/ml; Richardson (1974), about 3 ng/ml infused i.v. per min); and increases in CFC are indicative of increases in functional exchange vessel area, capillary permeability, or a combination of the two. In accordance with the present results, however, in skeletal muscle, isoprenaline (about 10 ng/ml i.a.) has been shown by Diana (1970) to increase capillary filtration coefficient whilst 1 μg/min intra-arterially does not increase lymph flow (Lewis & Winsey, 1970).

It has been suggested (Folkow *et al.*, 1963; Richardson, 1974), that isoprenaline increases capillary surface area and therefore the capillary filtration coefficient, yet the present study shows that the permeability surface area product is not increased. There are at least three possible explanations for this observation: (i) the lymphatics may be contracted by isoprenaline (Orlov, Borisova & Mundriko, 1976) and if the lymph flow was obstructed the PS product calculated in these experiments would decrease. However, fluid would accumulate on elevation of venous pressure and so the CFC would be increased by isoprenaline; (ii) if the net filtration pressure decreases, there must be a net reduction in capillary hydrostatic pressure since changes in tissue hydrostatic pressure, tissue or plasma oncotic pressures would be unlikely to explain a reduction in net filtration pressure. A fall in capillary hydrostatic pressure would be a consequence of an increase in precapillary:postcapillary resistance ratio which would be a result of a proportionately greater postcapillary dilatation than precapillary dilatation with isoprenaline. Such a contention is contrary to Diana's (1970) observation in skeletal muscle, though the β_2-adrenoceptor distribution is known to differ between tissues; (iii) the capillary filtration coefficients were determined by elevating the venous pressure from about 0 to 10 mmHg and over this pressure range, it is established that there is a 'myogenic' reduction in CFC (Johnson & Hanson, 1966; Mortillaro & Taylor, 1976). Because of the differences in the methods of determining PS and CFC, and particularly if isoprenaline interferes with these 'myogenic' effects (Mellander, 1978), there would be a greater 'capacity' for the CFC to increase with isoprenaline than for the PS to increase.

The low dose of bradykinin produced arterial concentrations about 40 ng/ml, similar to those shown by Fasth & Hulten (1973) to increase the capillary filtration coefficient. The present experiments show that the capillary filtration rate (= lymph flow in the steady state) and PS product both increase, yet the capillary permeability assessed by the measurement of the osmotic reflection coefficient (σ) is unaltered. This leads to the conclusion that the cause of the increase in filtration in the present experiments, and in the CFC in Fasth & Hulten's (1973) experiments was an increase in the capillary surface area, and not in capillary permeability.

The high doses of bradykinin (about 0.7 μg/ml) produced very large changes in filtration rate (lymph flow increased 15 times) and in the PS product (increased about 30 times). However, in these experiments, the calculated value for σ was decreased, indicating a marked increase in capillary permeability. In view of the effects of the lower concentrations of bradykinin, these increases in capillary permeability were probably accompanied by increases in capillary surface area. That bradykinin at these concentrations and above, increases capillary permeability in a variety of tissues is well-established (Lewis & Winsey, 1970; Joyner, Carter, Raizes & Renkin, 1974), though there has not hitherto been unequivocal evidence that bradykinin increases capillary surface area.

If bradykinin is released pathophysiologically (Seki, Nakajima & Erdos, 1972) from the gastrointestinal tract, the effects at low concentrations may be to increase transcapillary fluid movement by an increase in capillary surface area; only at much higher concentrations would there be increases in capillary permeability. The present experiments show that changes in capillary surface area may be distinguished from changes in capillary permeability due to drug infusions. The technique described in the present paper may help to differentiate between such mechanisms which are known to increase the intestinal capillary filtration coefficient in response to a number of drugs and hormones (Folkow *et al.*, 1963; Richardson, 1974; 1975; 1976; Granger *et al.*, 1978; 1979).

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