Br. J. Pharmac. (1978), **63**, 541–549

THE EFFECT OF ERGOTAMINE ON TISSUE BLOOD FLOW AND THE ARTERIOVENOUS SHUNTING OF RADIOACTIVE MICROSPHERES IN THE HEAD

BARBARA M. JOHNSTON[1] & P.R. SAXENA

Department of Pharmacology, Erasmus University Medical School, Rotterdam, The Netherlands

1 The radioactive microsphere method was used to study the effects of ergotamine (5, 10 and 20 μg/kg, i.v.) on systemic and regional haemodynamic variables in chloralose-urethane anaesthetized cats. The influence of the drug was also studied on the number of 15 μm microspheres escaping entrapment in the head to emerge in the left external jugular vein.

2 Ergotamine decreased the heart rate and cardiac output. Since arterial blood pressure remained unchanged, calculated total peripheral resistance increased.

3 The regional distribution of cardiac output obtained with 15 μm microspheres agreed well with previous studies in cats where 25 μm spheres were used. The most pronounced difference was that in the present investigation more microspheres, apparently escaping through arteriovenous anastomoses (AVAs), were detected in the lungs than when larger spheres had been used.

4 Coronary blood flow decreased, while uterine blood flow was increased by the drug. The microsphere content of the lungs, which receive the spheres not only via bronchial arteries but also via AVAs, was greatly reduced by all doses of ergotamine. Ergotamine did not influence tissue blood flow to other major organs such as the brain, kidneys, skin, liver, skeletal muscle or the gastrointestinal tract.

5 In the 16 experiments, 0.46 ± 0.05 (s.e. mean) % of the total microspheres injected (equivalent to 11.7 ± 1.4% of microspheres detected in the left-side of the head) appeared within 2 min of microsphere injection into the left external jugular vein. The highest dose of ergotamine significantly reduced the shunting of the microspheres in the head.

6 Since 15 μm microspheres are only likely to reach the lungs by passing into the venous circulation through large glomus-type AVAs, we conclude that ergotamine reduces the fraction of microspheres appearing in the lungs by causing strong vasoconstriction in the AVAs in the head.

7 In conformity with the closure of head AVAs is the finding that ergotamine reduced the jugular venous Po_2 and O_2 saturation thereby increasing the A-V O_2 saturation difference.

8 It is quite possible that decreased A-V shunting may be the prominent mechanism of the anti-migraine action of the drug, since sudden opening of AVA's has been implicated in the pathophysiology of migraine-syndrome.

Introduction

Ergotamine has long been the drug of choice for the treatment of acute attacks of migraine. However, its mechanism of action, like the pathophysiology of the migraine syndrome itself, is not completely understood. Classical migraine is characterized by an initial prodromal phase in which there is cerebral vasoconstriction which gives rise to non-painful sensory disturbances. This is followed by usually unilateral headache, probably caused by vasodilatation of the carotid artery, particularly the extracerebral branches of the external carotid artery (Dalessio, 1972). It has further been suggested (Heyck, 1958; 1969; Rowbotham & Little, 1965) that migraine attacks may result from the sudden opening of arteriovenous anastomoses (AVAs) in the head region; this idea would explain certain observations in the migraine syndrome, e.g. the extreme pallor of the skin in the presence of caro-

[1] Present address: Nuffield Institute for Medical Research, University of Oxford, Oxford.

tid vasodilatation, the increased prominence of scalp arteries and veins, and the finding that the oxygen saturation of external jugular and frontal venous blood approaches a level similar to that of arterial blood during migraine headache (Heyck, 1969).

Previous studies from this laboratory, with electromagnetic flowmeters in dogs, have shown that although ergotamine causes a general vasoconstriction in many vascular beds, this effect is particularly pronounced in the carotid and femoral arteries (Saxena & de Vlaam-Schluter, 1974) which supply areas having considerable AVAs (Gillilan & Markesbery, 1963; Rowbotham & Little, 1965; Lopez-Majno, Migita, Rhodes, Bardfeld & Wagner, 1971; Spence, Rhodes & Wagner, 1972).

Studies with electromagnetic flowmeters are necessarily restricted to observing flow through a limited number of vessels. Moreover, these flowmeter measurements can neither distinguish between 'nutrient' (capillary) and 'non-nutrient' (AVA) flow nor can they provide information about distribution of blood flow to the different structures supplied by a vessel. We have, therefore, used the radioactive microsphere method (Rudolph & Heymann, 1967), to measure the effects of ergotamine on the complete distribution of cardiac output, with particular reference to its effects on regional blood flow in the various tissues of the head. Although only a limited number of measurements of blood flow can be made, the microsphere method has the advantage that not only is it possible to measure flow to all tissues simultaneously, but by sampling the venous drainage of a particular region it is possible to obtain an index of flow through AVAs (Hales, 1974; Heymann, Payne, Hoffman & Rudolph, 1977) and the effects which particular drugs may have on these 'non-nutrient' vessels.

Methods

Cats of either sex (mean weight 2.9 kg) were anaesthetized with an intraperitoneal injection of chloralose 60 mg/kg and urethane 700 mg/kg. The trachea was cannulated and intermittant positive pressure ventilation (with the Harvard ventilation graph, Kleinmann & Radford, 1964) was continued throughout the experiment. The chest was opened on the left side by an incision between the 3rd and 4th ribs, and a 1.02 mm o.d. polyvinyl catheter was tied into the left atrium. Since we were particularly interested in blood flow to the head, it was considered important that the right common carotid artery should not be obstructed, as would be the case if the left ventricle was cannulated. A 6 mm electromagnetic flow probe (Skalar, Delft) was placed around the ascending aorta. The chest was closed and negative pressure restored. Catheters were placed in one femoral artery and vein for blood pressure measurement and drug administration, respectively. The left jugular vein was cannulated retrogradely with a 1.02 mm i.d. cannula so that the venous blood draining the head could be sampled during microsphere injection. The body temperature of the animals was maintained around 37°C and the ambient temperature was between 19–21°C.

Blood pressure and aortic flow were recorded for about 45 min after completion of the surgical procedures, and then a baseline measurement of cardiac output distribution was made by the radioactive microsphere technique (Rudolph & Heymann, 1967). The subsequent microsphere injections were made at 30 min intervals in all animals. Eight experimental animals were given intravenous doses of ergotamine (5,5 and then 10 μg/kg in 2 ml 0.9% w/v NaCl solution (saline); cumulative doses 5, 10 and 20 μg/kg) 10 min after each of the first three microsphere injections; thus the 2nd, 3rd and 4th batches of microspheres enabled changes in cardiac output distribution to be measured 20 min after administration of each dose of ergotamine. Eight control animals were given 2 ml saline 10 min after each of the first 3 microsphere injections. The principles and validation of the microsphere technique have been described in detail elsewhere (Rudolph & Heymann, 1967; Hales, 1974; Johnston, 1976; Heymann *et al.*, 1977). A suspension of approximately 80,000 to 150,000 of microspheres 15 ± 5 (s.d.) μm in diameter, labelled with ^{125}I, ^{141}Ce, ^{51}Cr or ^{85}Sr in 1 ml saline containing a drop of Tween 80, was ultrasonicated and then injected, each time in random order of isotope label, into the left atrium during a 15 to 20 s period. In order to demonstrate the possible effects of ergotamine on A-V shunting in the head, left jugular venous blood was collected for 2.5 min starting about 30 s before each microspheres injection. About 2 min before each batch of microspheres was given, systemic arterial and left jugular venous blood samples were taken for blood gas analysis in order to measure possible changes in arterio-venous oxygen saturation differences.

At the end of each experiment the animals were killed with an overdose of sodium pentobarbitone. The various organs and tissues were removed and weighed and their radioactivity measured in a Packard γ-scintillation counter equipped with a multichannel analyser using suitable windows for discriminating the isotopes used. The total radioactivity was measured in all organs except skin, body muscle and carcase, where aliquots of 25 to 33% were taken, from which their total radioactivity was computed. 'Carcase' refers to the remaining tissues when all organs had been removed and the body muscle scraped off (i.e. mainly bones, fat, and tail).

Calculations

The data were processed by PDP 11 computer using the spectral distribution stripping technique (see Heymann *et al.*, 1977). The fraction of cardiac output received by each tissue at each measurement period was calculated as the ratio of radioactivity in that tissue (TR) to the total radioactivity injected (TD). The total amount of radioactivity injected was determined both by summing the total counts in each tissue, and in 14 experiments additionally by the difference in counts in the syringe before and after each microsphere injection. The correlation between total doses obtained in the two ways was good ($r = 0.9891$, $n = 56$).

Cardiac output (CO) was calculated from aortic flow (AF) as measured by the electromagnetic flowmeter in ml/min and the percentage of microspheres trapped in the heart (PH): $CO = (AF \times 100)/(100 - PH)$. The flowmeter probe had been previously calibrated with several simultaneous measurements of CO by dye-dilution method. The end diastolic part of the flow signal was regarded as zero-flow line.

Tissue blood flows were calculated in $ml.min^{-1}.100\,g^{-1}$ (FL) by the formula: $FL = TR \times CO \times WT/(TD \times 100)$. WT represents the weight of the tissue in grams.

Tissue vascular resistance ($mmHg.ml^{-1}.min.100\,g$) was calculated by dividing mean arterial blood pressure, mmHg, by tissue blood flow, $ml.min^{-1}.100\,g^{-1}$. The central venous pressure was assumed to be negligible (as compared to mean arterial pressure) during the entire course of the experiment.

The percentage of cardiac output shunted in the left jugular vein was calculated by multiplying by 100 the ratio of jugular venous radioactivity (JR) and total radioactivity injected (TD).

Arteriovenous (A-V) shunt flow in the left jugular vein (AVF) was calculated as: $AVF = (JR/TD) \times CO$.

Percentage changes from baseline variables were calculated in each experiment and the changes obtained after each dose of ergotamine were compared with those obtained after corresponding saline injection. Due to the skewed distribution and lack of homogeneity of variance in some variables, the non-parametric two-tailed Mann Whitney U test (Siegel, 1956) was used for statistical evaluation.

Results

Ergotamine had no effect on blood pressure, but caused a decrease in heart rate which led to a reduction in cardiac output and consequent rise in total peripheral resistance, as shown in Table 1.

The mean baseline values for tissue blood flow, tissue resistance and cardiac output distribution to the

Table 1 Baseline values (mean ± s.e. mean) of systemic haemodynamic variables and changes in the groups of cats receiving saline or ergotamine

	Baseline measurements (n = 16)	*Saline control† (n = 24)*	*% changes after ergotamine (μg/kg, i.v.) 5 (n = 8)*	*10 (n = 8)*	*20 (n = 8)*
Aortic pressure (mmHg)					
Systolic	131 ± 5	−2 ± 4	−1 ± 3	0 ± 5	0 ± 6
Diastolic	78 ± 3	−8 ± 3	−3 ± 4	−2 ± 6	0 ± 7
Mean	96 ± 3	−4 ± 3	2 ± 4	−1 ± 6	0 ± 6
Heart rate (min^{-1})	214 ± 7	8 ± 2	−18 ± 3***	−20 ± 2***	−21 ± 2***
Cardiac index ($ml.min^{-1}.kg^{-1}$)	152 ± 9	−4 ± 3	−13 ± 5	−19 ± 3*	−24 ± 4*
Stroke volume (ml)	2.07 ± 0.13	−11 ± 2	7 ± 6	−2 ± 4	−3 ± 5
Total systemic resistance (units)§	0.67 ± 0.05	0 ± 2	15 ± 8	23 ± 9**	34 ± 11***

† The effects of three saline injections in the 8 cats were combined; § resistance = BP (mmHg)/cardiac index ($ml.min^{-1}.kg^{-1}$).
* $0.05 > P > 0.02$; ** $0.02 > P > 0.002$; *** $P < 0.002$.

various tissues are shown in Table 2. They are generally similar to those which were previously reported (Johnston & Owen, 1977) although a mean of nearly 10% of the cardiac output reached the lungs. This probably reflects a greater degree of systemic arteriovenous shunting since 15 μm microspheres were used in this study instead of the 25 μm microspheres used by Johnston & Owen (1977). The precision of the microsphere technique in our hands is substantiated by comparison of the (mean ± s.e. mean; $n = 16$) blood flow values ($ml.min^{-1}.100g^{-1}$) of identical organs. For example, both left and right kidneys received the same amount of blood (382 ± 37) and the left and the right ear received 3.44 ± 0.43 and 3.77 ± 0.44, respectively. The blood flow ($ml.min^{-1}.100g^{-1}$) distribution within 5 different regions of the brain was: left cerebral hemisphere (46 ± 5), right cerebral hemisphere (47 ± 5), diencephalon (48 ± 5), pons-medulla (51 ± 5) and cerebellum (60 ± 6). Furthermore, as can be expected the atria (158 ± 17) received a smaller blood flow ($ml.min^{-1}.100g^{-1}$) than the right ventricle (233 ± 23), atrioventricular septum (263 ± 22) or left ventricle (280 ± 28).

The effects of each of the three doses of ergotamine on cardiac output distribution, blood flow and resistance in selected tissues are shown in Table 3. The most notable effect was a progressive decrease in the fraction of cardiac output reaching the lungs. Since the radioactivity in the lungs represents both the microspheres entering with the bronchial arterial flow, and those not trapped in the capillary beds, it is likely that this observation indicates that ergotamine causes a reduction in the numbers of microspheres passing through the AVAs throughout the systemic circulation.

Ergotamine also caused a decrease in flow and fraction of cardiac output to the heart, with a corresponding rise in coronary resistance at all 3 doses. It is not clear from these experiments whether this is a cause or a consequence of the significant bradycardia resulting from drug administration (Table 1). Apart from the stomach, pancreas and liver receiving an increased fraction of cardiac output (not flow), the only other tissue in which ergotamine caused a significant haemodynamic response when compared with the control, was the uterus. All three doses of the drug increased the blood flow and the percentage of cardiac output distributed to uterus and decreased the tissue resistance.

The effects of ergotamine on regional blood flow

Table 2 Baseline values (mean ± s.e. mean) of regional haemodynamic variables in 16 cats

Organ	% *Cardiac output*	*Blood flow* ($ml.min^{-1}.100g^{-1}$)	*Resistance*† *units*
Lungs§	9.7 ± 1.2	261 ± 35	0.47 ± 0.07
Stomach	0.8 ± 0.06	14 ± 1	8.88 ± 1.78
Small intestine	7.8 ± 0.5	45 ± 4	2.38 ± 0.23
Large intestine	2.7 ± 0.3	66 ± 9	2.06 ± 0.40
Spleen	2.8 ± 0.5	225 ± 43	0.69 ± 0.12
Pancreas	0.4 ± 0.06	32 ± 6	4.86 ± 1.05
Liver*	17.9 ± 1.2	105 ± 8	1.02 ± 0.12
Adrenals	0.8 ± 0.08	1238 ± 372	0.12 ± 0.01
Body skin	2.2 ± 0.2	3.4 ± 0.4	35.50 ± 5.08
Body muscle	7.8 ± 1.1	3.8 ± 0.3	28.18 ± 2.78
Uterus**	0.2 ± 0.05	57 ± 22	4.92 ± 1.79
Heart	4.6 ± 0.3	253 ± 24	0.42 ± 0.03
GIT	11.3 ± 0.8	39 ± 3	2.74 ± 0.26
Portal***	14.6 ± 1.1	47 ± 5	2.37 ± 0.25
Total liver	32.5 ± 1.5	190 ± 13	0.54 ± 0.04
Kidneys	16.9 ± 1.4	382 ± 37	0.28 ± 0.03
Brain	3.1 ± 0.3	49 ± 5	2.24 ± 0.23
Head skin	0.5 ± 0.03	5.6 ± 0.5	20.06 ± 2.32
Head muscle	0.6 ± 0.05	5.4 ± 0.4	19.68 ± 2.09
Eyes	1.1 ± 0.09	48 ± 4	2.22 ± 0.17
Skull	1.6 ± 0.14	15 ± 1	7.45 ± 0.99
Ears	0.15 ± 0.02	3.6 ± 0.4	30.83 ± 2.75
Extracerebral	4.0 ± 0.2	11 ± 1	9.64 ± 0.78
Total head	7.1 ± 0.4	16 ± 1	6.45 ± 0.60

† Resistance = BP (mmHg)/blood flow ($ml.min^{-1}.100g^{-1}$); § bronchial + AVA flow; * hepatic artery flow; ** $n = 11$; *** the % CO and flow values were calculated by the addition of respective values of spleen, pancreas and GIT.

Table 3 Percentage changes (± s.e. mean) from baseline measurements by ergotamine in the haemodynamic variables of selected organs (8 cats)

	% changes by ergotamine (μg/kg., i.v.)								
	% *Cardiac output*			*Blood flow*			*Resistance*		
	5	10	20	5	10	20	5	10	20
Lungs†	−49 ± 6***	−63 ± 5***	−82 ± 2***	−57 ± 4***	−70 ± 3***	−87 ± 2***	142 ± 25***	261 ± 34***	762 ± 122***
Stomach	15 ± 5	35 ± 6*	54 ± 9*	1 ± 8	9 ± 7	19 ± 12	1 ± 8	−7 ± 7	−11 ± 9
Small intestine	3 ± 5	11 ± 4	29 ± 4	−10 ± 8	−10 ± 4*	−1 ± 6	15 ± 11	11 ± 7	4 ± 8
Large intestine	14 ± 8	24 ± 8	25 ± 7	−1 ± 9	0 ± 6	−5 ± 7	3 ± 8	1 ± 7	9 ± 9
Spleen	3 ± 26	11 ± 41	−7 ± 38	−8 ± 24	−6 ± 38	−23 ± 36	59 ± 33	106 ± 41	176 ± 58
Liver‡	12 ± 4*	15 ± 5*	18 ± 8*	−1 ± 8	−8 ± 4	−10 ± 7	4 ± 9	8 ± 6	15 ± 9
Body skin	9 ± 4	18 ± 6	22 ± 10**	−5 ± 6	−5 ± 6	−6 ± 10	6 ± 6	7 ± 9	14 ± 11
Body muscle	24 ± 10	27 ± 10	46 ± 12	9 ± 12	3 ± 11	12 ± 14	−3 ± 9	0 ± 7	−4 ± 10
Uterus§	48 ± 8**	66 ± 24**	85 ± 22***	15 ± 9*	31 ± 21*	36 ± 21**	−13 ± 12*	−17 ± 17*	−17 ± 24*
Heart	−21 ± 8*	−21 ± 8*	2 ± 8	−30 ± 10*	−29 ± 7***	−23 ± 6***	54 ± 16*	46 ± 14***	32 ± 7***
Kidneys	14 ± 6	28 ± 9	28 ± 7	0 ± 7	4 ± 9	−2 ± 9	4 ± 10	5 ± 15	10 ± 15
Brain	10 ± 3	23 ± 5	46 ± 9	−4 ± 5	0 ± 7	13 ± 12	3 ± 6	1 ± 8	−4 ± 12
Pancreas	24 ± 11*	37 ± 10*	48 ± 6*	9 ± 12	13 ± 10	14 ± 7	−4 ± 10	−8 ± 9	−10 ± 7
Adrenals	23 ± 5	23 ± 8	15 ± 15	8 ± 10	1 ± 10	−11 ± 14	5 ± 15	13 ± 15	45 ± 30

† Bronchial + A-V shunt flow; ‡ hepatic artery + portal flow; § $n = 5$.
* $0.05 > P > 0.02$; ** $0.02 > P > 0.002$; *** $P < 0.002$: comparison was made with the changes occurring during corresponding saline injection. These are not shown in this table.

to the different extracerebral regions of the head (skin, muscle, eyes, skull and ears) were also studied. No effect was noticeable on any tissue except the skull where the two highest doses (10 and 20 μg/kg) caused some reduction of blood flow (-38 ± 8 and $-44 \pm 5\%$) and increase in tissue vascular resistance (73 ± 18 and $88 \pm 22\%$).

Table 4 shows the effects of ergotamine on the index of A-V shunting in the left external jugular vein. At the baseline $0.46 \pm 0.05\%$ (s.e. mean, $n = 16$) of the total number of microspheres injected in the left atrium (or $11.7 \pm 1.4\%$ of the microspheres trapped in the left half of head) escaped entrapment by the tissue capillaries and were detected in the left external jugular vein. At the highest dose (20 μg/kg, i.v.), ergotamine significantly reduced the appearance of microspheres in the venous drainage of head. No change was observed in body temperature (Table 4).

Table 5 shows the effect of ergotamine on a number of blood chemistry variables. The three doses (5, 10 and 20 μg/kg) of ergotamine used in this investigation increased the difference (normally 9.1%) in O_2-saturation between the peripheral arterial and left jugular venous bloods by 114, 180 and 261%, respectively. Although in the saline group the A-V O_2 saturation difference also increased by 57%, due probably to partial occlusion of AVAs by the microspheres, the values obtained after ergotamine administration were significantly higher. Since ergotamine had little influence on arterial Po_2 and O_2 saturation but did decrease these variables of the venous blood the effect of the drug on A-V O_2 saturation difference was due

Table 4 Effect of ergotamine on arteriovenous anastomosis (AVA) flow in left external jugular vein and on the body temperature of cats

	Baseline values $n = 16$	*Saline control* $n = 24$	% *change by ergotamine (μg/kg, i.v.)* 5 $n = 8$	10 $n = 8$	20 $n = 8$
% Shunting†	0.46 ± 0.05	−29 ± 5	−37 ± 14	−49 ± 10	−68 ± 11**
Shunt flow§ (ml/min)	1.94 ± 0.22	−32 ± 5	−44 ± 13	−59 ± 8	−76 ± 8**
Body temperature	37.2 ± 0.2	2 ± 0.5	1 ± 0.3	2 ± 1	2 ± 1

Values are mean ± s.e. mean; † % of cardiac output shunted in left jugular vein; § A-V shunt flow in left jugular vein.
** $0.02 > P > 0.002$.

Table 5 Baseline value (mean ± s.e. mean) of blood chemistry and changes in the groups of cats receiving saline or ergotamine

	Baseline values ($n = 16$)	*Saline control* ($n = 24$)	% *Change after ergotamine (μg/kg, i.v.)* 5 ($n = 8$)	10 ($n = 8$)	20 ($n = 8$)
A-V O_2 saturation difference (%)	9.1 ± 1.0	57 ± 19	114 ± 25*	180 ± 30*	261 ± 42***
Arterial blood					
pH	7.32 ± 0.01	0 ± 0.1	0 ± 0.1	0 ± 1	0 ± 0.2
Pco_2 (mmHg)	31 ± 1	0 ± 3	−6 ± 1	−6 ± 2	−5 ± 2
Po_2 (mmHg)	89 ± 3	−4 ± 1	4 ± 2	0 ± 2	−6 ± 3
O_2 saturation (%)	95 ± 1	−1 ± 0.3	1 ± 0.3	0 ± 0.4	−1 ± 0.3
Venous blood					
pH	7.28 ± 0.01	0 ± 0.1	0 ± 0.1	−1 ± 0.1	−1 ± 0.2
Pco_2 (mmHg)	33 ± 1	1 ± 2	4 ± 3	10 ± 3	15 ± 5
Po_2 (mmHg)	60 ± 1	−8 ± 3	−16 ± 3	−24 ± 4*	−31 ± 4*
O_2 saturation (%)	86 ± 1	−5 ± 2	−8 ± 3	−14 ± 2*	−22 ± 2***

* $0.05 > P > 0.02$; ** $0.02 > P > 0.002$; *** $P < 0.002$.

to closure of AVAs in the head. However, increased O_2 extraction by the tissues may also be involved to some extent.

Discussion

As reported previously in dogs (Saxena & de Vlaam-Schluter, 1974), ergotamine lowered the heart rate and cardiac output in cats. These effects may result from peripheral vasoconstriction and/or stimulation of presynaptic α-adrenoceptors (Langer, 1977) leading to decreased noradrenaline output from the nerve terminals. The capacity of ergotamine to excite α-adrenoceptors has been noted in several studies (Innes, 1962; Saxena & de Vlaam-Schluter 1974; Müller-Schweinitzer & Stürmer, 1974) and the drug can also inhibit reflex vascular responses at the peripheral neuroeffector site (Wellens, Szigetvari & Wauters, 1970). The regional vascular effects of ergotamine were such that the cardiac output was preferentially distributed to stomach, liver, uterus and pancreas at the expense of lungs (bronchial + A-V shunt flow) and heart. Due to a reduction in cardiac output, only uterine blood flow increased. Although ergot derivatives are used to prevent post-partum placental bleeding, probably by compression of arterioles by an oxytocic action, the effect of these drugs on uterine blood flow had not been studied (Woodbury, 1971). It is possible that the increased uterine blood flow demonstrated in this study is secondary to enhanced metabolic demands initiated by contraction of uterus by ergotamine.

The appearance of particles of known size, such as glass beads, in the venous blood has been used for a long time for the study of glomus-type AVAs. However, the difficulty in counting procedure and the non-uniformity of the size of the particles have sometimes produced equivocal results. The availability of plastic microspheres labelled with radionuclide has overcome these difficulties and many investigators have used such microspheres for the study of AVAs (for references, see Hales, 1974; Heymann *et al.*, 1977; Hales, Fawcett, Bennett & Needham, 1978). Since, the diameter of AVAs (15 to 150 μm) is generally greater than that of the arterioles (20 to 30 μm) or capillaries (1 to 8 μm) (Clark & Clark, 1934; Burton, 1944; Daniel & Prichard, 1956; Sherman, 1963), the use of 15 μm, in contrast to large size microspheres, provides a preferential measure of 'nutrient' (capillary) blood flow to the organs (Hales, 1974). It has also been demonstrated that the 15 μm microspheres are almost completely trapped by the arterioles and capillaries in the lungs (99 to 100%), as well as in the tissues where the large-sized AVAs are scarce, e.g. skeletal muscle, kidney, heart, brain or gastrointestinal tract (Slotkoff, Logan, Jose, D'Avella & Eisner, 1971; Archie, Fixler, Ullyot, Hoffman, Utley & Carlson, 1973; Hales, 1974; Warren & Ledingham, 1974; Marcus, Heistad, Ehrhardt & Abboud, 1976; Zinner, Kerr & Reynolds, 1976). There is considerable shunting of 15 μm microspheres in skin, carotid (mainly extracerebral part), femoral and ear circulation (Lopez-Majno *et al.*, 1971; Spence *et al.*, 1972; Hales, 1974; Warren & Ledingham, 1974), where the AVAs are extensively distributed not only in animals but also in man (Clara, 1956; Daniel & Prichard, 1956; Gillilan & Markesbery, 1963; Rowbotham & Little, 1965). The cardinal point, that spheres of this size do not escape to the venous side through capillaries in substantial number, was demonstrated by Hales & Cliff (1975; 1977) who followed the behaviour of acutely injected microspheres by cinematography in surgically prepared rabbit ear chambers. They found that microspheres of 15 μm diameter were already trapped in the end-arterioles and precapillary sphincter regions of the microvasculature. Similarly, Alm (1975) has found that a considerable number of 35 μm microspheres was lodged after an intracardiac injection in the large pial arteries of the monkey. It appears that the microspheres, being slightly heavier than the blood, require higher pressure than the blood cells for onward propulsion. Thus, the 15 μm, microsphere reach the venous blood only through large-sized glomus-type AVAs and ultimately stack themselves in the pulmonary vessels. Since bronchial arterial blood flow, as determined by large sized (50 μm) microspheres, is generally less than 1.5% of CO (Forsyth, Nies, Wyler, Neutze & Melmon, 1968) the amount of microspheres detected in the lungs (about 10%) represents mainly the blood flowing through the AVAs. Similarly, the microsphere content of external jugular venous blood is an index of AVA-flow in the head regions, most likely the extracerebral tissues and not so much the brain itself (Hales, 1972; Marcus *et al.*, 1976).

Since ergotamine greatly decreased the microsphere content of the lungs, it appears that the drug reduced the shunting of blood through the AVA's. More direct evidence is, of course, provided by the fact that significantly fewer microspheres appeared in the external jugular vein after 20 μg/kg dose of the drug than after the corresponding saline injection. As Creasy, Kahanpaa & De Swiet (1974) have shown by external counting that the microspheres may continue to appear in venous circulation for up to 15 min, it is possible that the venous withdrawal period (2 min) used by us may apparently be a less sensitive method for studying the drug effect; 15 to 20 min withdrawal time when using 4 microsphere labels is for obvious reasons impracticable. Nevertheless, we have recently obtained data (unpublished) showing that about 40% of 15 μm microspheres injected in the common carotid artery traverse through AVAs, probably in the

rete-mirabile conjugatum (Gillilan & Markesbery, 1963), nasal mucosa, skull or the head skin, to appear in the lungs. Ergotamine, in intravenous doses of 5, 10 and 20 μg/kg, significantly reduced this number to 23, 13 and 5%, respectively. In conformity with ergotamine's ability to close the AVAs in the head region is the fact that the A-V oxygen saturation difference was increased by the drug. Closure of AVAs would decrease the contribution to the venous side of the arterial blood not undergoing O_2-loss in the capillary bed. The effects of ergotamine were independent of any influence of temperature on the AVAs (Zanick & Delaney, 1973; Hales *et al.*, 1978) and it may be recalled that, even in electromagnetic flowmeter studies, the most prominent effect of the drug was found on the carotid and femoral vascular bed (Saxena & de Vlaam-Schluter, 1974) where there is evidence of abundant AV-shunting.

Finally, attention should be drawn to the fact that 5-hydroxytryptamine (5-HT; serotonin), one of the biogenic amines implicated in the pathophysiology of migraine, has the ability to dilate arterioles and to constrict large arteries (Haddy, 1960) and, probably also closes AVAs (Rondell, Palmer & Bohr, 1957). Since the cerebral blood vessels are far more sensitive to 5-HT (Toda & Fujita, 1973), it is conceivable that a decline of blood 5-HT concentrations in the headache phase of migraine (Anthony, Hinterberger & Lance, 1969; Sommerville, 1976) may dilate the large arteries and constrict the arterioles, a situation that would lead to increased AVA flow and tissue hypoxia. As already pointed out, there is evidence to assume that migraine attacks are associated with sudden opening of AVAs in the head (Heyck, 1958; 1969; Rowbotham & Little, 1965). Our data demonstrating that the most effective antimigraine agent, ergotamine, constricts AVAs in the head without affecting cerebral blood flow and, in addition, increases A-V O_2 extraction, not only give credence to the aforementioned view regarding the pathophysiology of migraine syndrome but also suggest that the closure of AVAs is the most probable mechanism of the therapeutic action of the drug. It may be added that, especially if one keeps in mind the higher capacity of common laboratory animals than humans to metabolize drugs, the doses of ergotamine employed in the present investigation (5 to 20 μg/kg) compare favourably with that (0.50 mg or about 8 μg/kg) commonly used clinically.

B.M.J. was supported by a grant from the Research pool of the Faculty of Medicine, Erasmus University, Rotterdam, The Netherlands. The authors are grateful to Dr Ralph Forsyth (Cardiovascular Research Institute, University of California, San Francisco, San Francisco, Calif., U.S.A.) for his help in the initial stages of this investigation. We are also indebted to Dr J.R.S. Hales (C.S.I.R.O., Ian Clunies Ross Animal Research Laboratory, Prospect, N.S.W., Australia) for critically reading the manuscript. Ir. J. Loeve was helpful in the data analysis and the blood chemistry was done by Central Clinical Chemistry Labs (Head: Prof. B. Leijnse). Ergotamine tartrate was supplied by Messrs. Sandoz BV, Uden, The Netherlands through the courtesy of Mr P. Baan. Reprint requests to P.R.S., please.

References

ALM, A. (1975). Radioactively labelled microspheres in regional cerebral blood flow determinations. A study on monkeys with 15 and 35 μm spheres. *Acta physiol. scand.*, **95**, 60–65.

ANTHONY, M., HINTERBERGER, H. & LANCE, J.W. (1969). The possible relationship of serotonin to the migraine syndrome. *Res. Clin. Stud. Headache*, **2**, 29–59.

ARCHIE, J.P., FIXLER, D.E., ULLYOT, D.J., HOFFMAN, J.I.E., UTLEY, J.R. & CARLSON, E.L. (1973). Measurement of cardiac output with an organ trapping of radioactive microspheres. *J. appl. Physiol.*, **35**, 148–154.

BURTON, A.C. (1944). Relation of structure to function of the tissues of the wall of blood vessels. *Physiol. Rev.*, **34**, 619–642.

CLARA, M. (1956). *Die arterio-venösen Anastomosen.* 2nd Ed. Wien: Springer Verlag.

CLARK, E.R. & CLARK, E.L. (1934). Observations on living arterio-venous anastomoses as seen in transparent chambers introduced into the rabbit's ear. *Am. J. Anat.*, **54**, 229–286.

CREASY, R.K., KAHANPAA, K.V. & DE SWIET, M. (1974). Trapping of radioactive microspheres in the pregnant and non-pregnant rabbit. *Acta physiol. scand.*, **90**, 252–259.

DALESSIO, D.J. (1972). *Wolff's Headache and Other Head Pains.* 3rd. Ed. New York: Oxford University Press.

DANIEL, P.M. & PRICHARD, M.M.L. (1956). Arteriovenous anastomoses in the external ear. *Q. Jl. exp. Physiol.*, **41**, 107–123.

FORSYTH, R.P., NIES, A.S., WYLER, F., NEUTZE, J. & MELMON, K.L. (1968). Normal distribution of cardiac output in the unanaesthetized, restrained monkey. *J. Appl. Physiol.*, **25**, 736–741.

GILLILAN, L.A. & MARKESBERY, W.R. (1963). Arteriovenous shunts in the blood supply to the brain of some common laboratory animals—with special reference to the rete mirabile conjugatum in the cat. *J. comp. Neurol.* **121**, 305–311.

HADDY, F.J. (1960). Serotonin and the vascular system. *Angiology*, **11**, 21–24.

HALES, J.R.S. (1972). Chronic catheterization for sampling venous blood from the brain of the sheep. *Pflügers Arch.*, **337**, 81–85.

HALES, J.R.S. (1974). Radioactive microsphere techniques for studies of the circulation. *Clin. exp. Pharmac. Physiol.*, suppl. 1, 31–46.

HALES, J.R.S. & CLIFF, W.J. (1975). Behaviour of microspheres in micro-vasculature. Film produced by T.C. Dagg, C.S.I.R.O., Ian Clunies Ross Animal Research Laboratory, Sydney.

HALES, J.R.S. & CLIFF, W.J. (1977). Direct observations on the behaviour of microspheres in microvasculature.

Proc. IXth World Congress, European Microcirculation Society, Part 1, pp. 87–91.

HALES, J.R.S., FAWCETT, A.A., BENNETT, J.W. & NEEDHAM, A.D. (1978). Blood flow through capillaries and arteriovenous anastomoses in skin of sheep: microsphere technique and thermal control. *Pflügers Arch.*, (in press).

HEYCK, H. (1958). *Der Kopfschmerz*. p. 301. Stuttgart: Georg Thieme Verlag.

HEYCK, H. (1969). Pathogenesis of migraine. *Res. Clin. Stud. Headache*, **2**, 1–28.

HEYMANN, M.A., PAYNE, B.D., HOFFMAN, J.I.E. & RUDOLPH, A.M. (1977). Blood flow measurements with radionuclide labelled particles. *Progr. Cardiovasc. Dis.* **20**, 55–79.

INNES, I.R. (1962). Identification of the smooth muscle excitatory receptors for ergot alkaloids. *Br. J. Pharmac. Chemother.*, **19**, 120–128.

JOHNSTON, B.M. (1976). Redistribution of regional blood flows by some anti-hypertensive drugs. *D.Phil. Thesis, University of Oxford.*

JOHNSTON, B.M. & OWEN, D.A.A. (1977). Tissue blood flow and distribution of cardiac output in cats: changes caused by intravenous infusions of histamine receptor agonists. *Br. J. Pharmac.*, **60**, 173–180.

KLEINMANN, L.I. & RADFORD, E.P., Jr. (1964). Ventilation standards for small mammals. *J. appl. Physiol.*, **19**, 360–362.

LANGER, S.Z. (1977). Presynaptic receptors and their role in the regulation of transmitter release. *Br. J. Pharmac.*, **60**, 481–497.

LOPEZ-MAJANO, V., MIGITA, T., RHODES, B.A., BARDFELD, P. & WAGNER, H.N. (1971). Distribution of the carotid circulation in dog. *Cor Vasa*, **13**, 71–76.

MARCUS, M.L., HEISTAD, D.D., EHRHARDT, J.C. & ABBOUD, F.M. (1976). Total and regional cerebral blood flow measurements with 7–10μ, 15μ, 25μ, and 50μ diameter microspheres. *J. appl. Physiol.*, **40**, 501–507.

MÜLLER-SCHWEINITZER, E. & STÜRMER, E. (1974). Investigations on the mode of action of ergotamine in the isolated femoral vein of the dog. *Br. J. Pharmac.*, **51**, 441–446.

RONDELL, P.A., PALMER, L.E. & BOHR, D.F. (1957). Influence of pharmacologic agents on capillary-AVA flow distribution in the perfused rabbit ear. *Fedn Proc.*, **16**, 109.

ROWBOTHAM, G.F. & LITTLE, E. (1965). New concepts on the aetiology and vascularization of meningiomata: the mechanism of migraine; the chemical process of cerebrospinal fluid; and the formation of collections of blood or fluid in the subdural space. *Br. J. Surg.*, **52**, 21–24.

RUDOLPH, A.M. & HEYMANN, M.A. (1967). The circulation of the fetus in utero: Methods for studying distribution of blood flows, cardiac output and organ blood flow. *Circulation Res.*, **21**, 163–184.

SAXENA, P.R. & DE VLAAM-SCHLUTER, G.M. (1974). Role of some biogenic substances in migraine and relevant mechanism in antimigraine action of ergotamine—studies in an experimental model for migraine. *Headache*, **13**, 44–54.

SHERMAN, J.L. (1963). Normal arteriovenous anastomoses. *Medicine*, **42**, 247–267.

SIEGEL, S. (1956). *Non Parametric Statistics for the behavioural Science*. Tokyo: McGraw-Hill.

SLOTKOFF, L.M., LOGAN, A., JOSE, P., D'AVELLA, A. & EISNER, G.M. (1971). Microsphere measurement of intrarenal circulation of the dog. *Circulation Res.*, **28**, 158–166.

SOMMERVILLE, B.W. (1976). Platelet bound and free serotonin levels in jugular and forearm venous blood during migraine. *Neurology*, **26**, 41–45.

SPENCE, R.J., RHODES, B.A. & WAGNER, H.N. (1972). Regulation of arteriovenous anastomotic and capillary blood flow in dog leg. *Amer. J. Physiol.*, **222**, 326–332.

TODA, N. & FUJITA, Y. (1973). Responsiveness of isolated cerebral and peripheral arteries to serotonin, and transmural electrical stimulation, *Circulation Res.*, **33**, 98–104.

WARREN, D.J. & LEDINGHAM, J.G.G. (1974). Measurement of cardiac output distribution using microspheres. Some practical and theoretical considerations. *Cardiovasc. Res.*, **8**, 570–581.

WELLENS, D., SZIGETVARI, E. & WAUTERS, E. (1970). Reflex vasodilation, ergotamine and uptake of circulatory norepinephrine. *Arch. int. Pharmacodyn. Ther.*, **183**, 412–415.

WOODBURY, R.A. (1971). Drugs acting on the uterus: pituitary hormones, ergot alkaloids, and prostaglandins. In *Drill's Pharmacology in Medicine*. 4th Ed. ed. Di Palma, J.R. p. 1423. New York: McGraw-Hill Book Company.

ZANICK, D.C. & DELANEY, J.P. (1973). Temperature influences on arteriovenous anastomoses. *Proc. Soc. exp. Biol. Med.*, **144**, 616–620.

ZINNER, M.J., KERR, J.C. & REYNOLDS, D.G. (1976). Distribution and arteriovenous shunting of gastric blood flow in the baboon: Effect of epinephrine and vasopressin infusions. *Gastroenterol.*, **71**, 299–302.

(Received January 6, 1978.
Revised February 16, 1978.)