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The influence of methysergide on 5-hydroxytryptamine-induced changes in regional distribution of blood flow

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Systemic and regional haemodynamic variables were measured at the baseline and after saline or 5-HT infusions (5 μg kg^{-1} min^{-1}, i.v.) or methysergide injections (0·5 mg kg^{-1}, i.v.). Cardiac output and its complete distribution were measured by the radioactive microsphere (15 μm diam.) technique. Although 5-HT did not change the systemic variables, methysergide caused a moderate increase in systolic and mean blood pressure and heart rate. 5-HT caused a substantial increase in gastric and a moderate increase in cerebral and myocardial blood flow at the expense of that to the lungs (arteriovenous shunt + bronchial flows), kidneys and skin. While methysergide was able to reduce the vascular responses to 5-HT in stomach, skin, kidneys, heart, lungs and brain, the drug itself, like 5-HT, decreased the number of microspheres reaching the lungs. Since a large number of 15 μm microspheres can escape through the arteriovenous anastomoses to lodge in the lungs it seems likely that both 5-HT and methysergide can reduce the 'non-nutrient' flow through these anastomoses.

Migrainous headaches are usually characterized by unilateral headaches associated with certain vascular changes in intra- and extracranial circulation (Dalessio, 1972). Although the exact cause is unknown it seems that one factor is a sharp decline in the concentration of 5-hydroxytryptamine (5-HT) in blood (Anthony, Hinterberger & Lance, 1969; Lance, 1973; Sommerville, 1976). However, the implication that migraine is a 'low 5-HT' syndrome does not relate to the effectiveness of methysergide, a potent anti-5-HT compound (Fanchamps, Doepfner & others, 1960), as an antimigraine agent. Indeed, there are indications that at least in some circumstances methysergide can have a pro-5-HT rather than anti-5-HT action (Saxena, 1972; 1974; Fozard, 1975; Apperley, Humphries & Levy, 1976). The present investigation, using the radioactive microsphere technique (Rudolph & Heymann, 1967; Hoffbrand & Forsyth, 1969), has examined the effects of methysergide on regional circulation and regional circulatory responses to 5-HT.

MATERIALS AND METHODS

The experiments used 11 New Zealand white rabbits, 2·2 to 4·0 kg, anaesthetized with sodium pentobarbitone (30 mg kg^{-1}, i.v.). A tracheotomy was performed and positive pressure ventilation applied with a Palmer respiratory pump at tidal volumes and frequency indicated by the Harvard Ventilation Graph (Kleinmann & Radford, 1964). Polyvinyl catheters were inserted into the abdominal aorta and the inferior vena cava via the left femoral artery and vein. The chest was then opened on the left side between the 2nd and 3rd ribs. The pericardium was incised and a 0·75 mm i.d. polyvinyl catheter was inserted and tied in the left atrium. After closing the chest 30–45 min of baseline recordings were made before the beginning of each experiment. The arterial blood pressure was measured with Statham transducers using a Grass model 7 polygraph.

The distribution of cardiac output to peripheral tissues was measured at four separate times in each rabbit, before and after each of the two drugs or saline infusions, using the radioactive microsphere method (Rudolph & Heymann, 1967). The principles and validation of the microsphere method have been described elsewhere (Hoffbrand & Forsyth, 1969; Hales, 1974; Heymann, Payne & others, 1977). At each measurement a suspension of approximately 80 000 to 100 000 microspheres of 15 $\pm$ 5 μm diam. mixed in 1 ml saline was injected into the left atrium and flushed with 2 ml saline over 15–20 s. A different batch of gamma emitting labelled microspheres (either ^{125}I, ^{141}Ce, ^{51}Cr or ^{85}Sr) was used at each measurement. In order to calculate cardiac output by the microsphere method, arterial blood was withdrawn at the rate of 5 ml min^{-1} through the aortic catheter for 60 s starting about 5–10 s before each microsphere injection. The first (baseline) microsphere measurement was made in animals before

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treatment. The second batch of microspheres was injected after the animals had received 20 min intravenous infusions (rate: 0·1 ml min^{-1}) of either 0·9% saline (n = 6) or 5-HT creatinine sulphate dissolved in 0·9% saline (5 μg kg^{-1} min^{-1}, as base; n = 5). Although the third microsphere measurement was made in all animals 15–20 min after an injection of methysergide (0·5 mg kg^{-1}, i.v.), only those rabbits (n = 6) which had earlier received saline were included for the analysis of the effect of methysergide. Finally, before the last microsphere injection, the animals received a 5-HT infusion as indicated above. At the end of the experiment the animals were killed with an overdose of pentobarbitone sodium and the various organs or tissues and blood samples, weighed and placed in vials and the amount of radioactivity (c min^{-1}) counted in a Nuclear Chicago Gamma Scintillation counter with pre-set windows discriminating the isotope energies. Whole tissues were counted except liver, skin, muscle, bones, small and large intestines when a 30–50% aliquot was used.

The percent of cardiac output received by each tissue at each measurement time was esimated as the ratio of the radioactivity in that tissue to the total amount of radioactivity injected $\times 100$. The total amount of the radioactivity injected for each nuclide label used was determined by summing the radioactivity (c min^{-1}) in all body tissues and a blood sample. Cardiac output (CO) was determined by the dye-dilution method and by the microsphere method using the formula: *CO* (ml min^{-1}) = blood withdrawal rate (ml min^{-1}) $\times$ total dose (c min^{-1}) of isotope injected divided by the (c min^{-1}) of that nuclide in the blood sample. Blood flow to each organ was calculated by multiplying the fraction of cardiac output by the cardiac output (obtained by dye-dilution method) and the resistance was calculated as the flow divided by the driving pressure. The change in each of the measured variables after 5-HT or methysergide was calculated for each animal and compared with those for saline. Also, comparison was made between the effects of 5-HT before and after the injection of methysergide. Due to the skewed distribution and lack of homogeneity of variance in some variables the non-parametric Mann-Whitney U test (Tate & Clelland, 1957) was used for statistical evaluation.

RESULTS

Effect on systemic variables

The baseline measurements of systematic haemodynamic variables and the changes caused by saline, methysergide and 5-HT are shown in Table 1. The methysergide-induced changes are from 6 animals which had previously only received saline infusions. Methysergide (0·5 mg kg^{-1}) caused statistically significant but moderate increases in systolic and mean blood pressure and in heart rate, while infusion of 5-HT (5 μg kg^{-1} min^{-1}) did not result in statistically significant changes when compared with saline.

The cardiac output values in Table 1 were obtained using the dye-dilution technique where blood was withdrawn at the rate of 8–10 ml min^{-1} during the dye injection. The cardiac output value in the animals using the microsphere method was found to be 338 $\pm$ 12 ml min^{-1} (s.e.m.; n = 41) compared with 369 $\pm$ 16 ml min^{-1} using the dye-dilution technique (correlation coefficient being 0·8723). The individual pairs of values are shown in Fig. 1.

Effect on regional circulation

The baseline values of regional distribution of cardiac output and blood flow (Table 2) were similar to those previously reported (Neutze, Wyler & Rudolph, 1968; Johnston, 1975) and to those we have found in our own recent experiments (Saxena, Forsyth & others, 1978). The precision of the micro-

Table 1. *Baseline values of systemic variables and changes induced by saline, methysergide or 5-HT.* A: Saline infusion (n = 6); B: methysergide (0.5 mg kg^{-1}, i.v.; n = 6); 5-HT (5μg kg^{-1} min^{-1}, i.v.) C: before methysergide (n = 5), D: after methysergide (n = 11).

Systemic variables	Baseline measurement n = 11	Mean ± s.e.m. % change induced by A	B	C	D
Systemic arterial pressure (mm Hg)					
Systolic	112 ± 4	−12 ± 5	9 ± 5*	−11 ± 3	−3 ± 4
Diastolic	71 ± 3	−10 ± 4	3 ± 6	−18 ± 4	−15 ± 3
Mean	84 ± 3	−12 ± 3	3 ± 5*	−13 ± 5	−9 ± 3
Heart rate (beats min^{-1}	279 ± 5	0 ± 3	8 ± 3*	−2 ± 4	1 ± 2
Cardiac output (ml min^{-1} kg^{-1})†	135 ± 8	−12 ± 3	1 ± 5	−10 ± 3	−5 ± 4
Total systemic peripheral resistance§	644 ± 46	1 ± 4	3 ± 6	−3 ± 5	−3 ± 5

† Determined by dye-dilution method.
§ Calculated by the formula Mean b.p. (mm Hg)/cardiac output (*litre* min^{-1} kg^{-1}).
* $P = <0.05$. Statistical comparison was done with saline (A) column; i.e. between B and A, C and A, and D and A (2-tailed Mann-Whitney U test).

Table 2. *Effects of methysergide and 5-HT on the regional distribution of cardiac output and blood flow in rabbits.* A: saline infusion (n = 6). B: methysergide (0·5 mg kg^{-1}, i.v.; n = 6). 5-HT (5 μg kg^{-1} min^{-1}, i.v.) C: before methysergide (n = 5), D: after methysergide (n = 11).

Tissue	Mean ± s.e.m. baseline % CO n = 11	Cardiac output % Mean ± s.e.m. % change induced by A	B	C	D	Mean ± s.e.m. baseline values; flow (ml min^{-1}/100 g) n = 11	Blood flow Mean ± s.e.m. % change induced by A	B	C	D
Lungs†	3·1 ± 0·3	3 ± 4	−32 ± 9*	−39 ± 5*	−12 ± 10**	97 ± 11	−9 ± 4	−32 ± 10*	−45 ± 5*	−15 ± 10**
Brain	1·1 ± 0·1	15 ± 4	35 ± 21	34 ± 8*	4 ± 5**	48 ± 3	1 ± 4	36 ± 22*	19 ± 4*	−3 ± 5**
Heart	3·2 ± 0·1	4 ± 2	44 ± 23*	54 ± 11*	1 ± 4**	230 ± 13	−9 ± 4	45 ± 24*	38 ± 10*	−5 ± 4**
Kidneys	19·2 ± 1·1	−3 ± 2	−7 ± 3	−29 ± 4*	−8 ± 4**	433 ± 29	−15 ± 1	−6 ± 5	−37 ± 3*	−13 ± 4**
Skin	6·4 ± 0·8	0 ± 5	−18 ± 8	−37 ± 4*	−7 ± 9**	10 ± 1	−11 ± 5	−17 ± 8	−44 ± 2*	−12 ± 5**
Skeletal muscle	16·7 ± 1·0	−4 ± 2	−4 ± 3	−14 ± 8	3 ± 5	6 ± 0·5	−15 ± 3	−3 ± 6	−24 ± 4	−2 ± 7
Stomach	3·8 ± 0·3	−1 ± 6	13 ± 12	418 ± 67*	81 ± 23*,**	55 ± 5	−11 ± 7	15 ± 14	368 ± 69*	68 ± 19*,**
Small intestine	9·2 ± 0·7	4 ± 3	16 ± 5*	1 ± 8	4 ± 2	98 ± 9	−8 ± 5	17 ± 7*	−9 ± 10	−1 ± 5
Large intestine	9·5 ± 0·5	5 ± 3	10 ± 8	−2 ± 12	0 ± 3	51 ± 3	−8 ± 3	12 ± 12	−11 ± 12	−5 ± 5
Spleen	1·1 ± 0·2	−9 ± 6	−9 ± 19	−34 ± 14	−21 ± 13	508 ± 101	−18 ± 8	−9 ± 19	−40 ± 13	−23 ± 15
Pancreas	0·4 ± 0·1	−16 ± 5	−14 ± 17	−35 ± 9	−16 ± 7	39 ± 5	−26 ± 5	−14 ± 17	−42 ± 8	−20 ± 7
Mesentery	1·0 ± 0·2	−14 ± 6	−16 ± 11	−27 ± 8	−7 ± 5	18 ± 3	−24 ± 6	−15 ± 11	−35 ± 6	−15 ± 7
Liver (arterial + portal)	29·1 ± 0·8	2 ± 1	7 ± 4	44 ± 14*	8 ± 3	123 ± 9	−10 ± 3	9 ± 8*	31 ± 16*	−13 ± 4
Ears	0·3 ± 0·04	−13 ± 4	31 ± 10*	−29 ± 18	9 ± 8*,**	5 ± 1	−24 ± 5	31 ± 10*	−38 ± 12	8 ± 6*,**

† Includes bronchial and A–V anastomotic blood flow.
* P = <0·05. Statistical comparison was done with saline (A) column; i.e. between B and A, C and A, and D and A (2-tailed Mann–Whitney U test),
** P = <0·05. Statistical comparison was done between columns D and C (2-tailed Mann–Whitney U test).

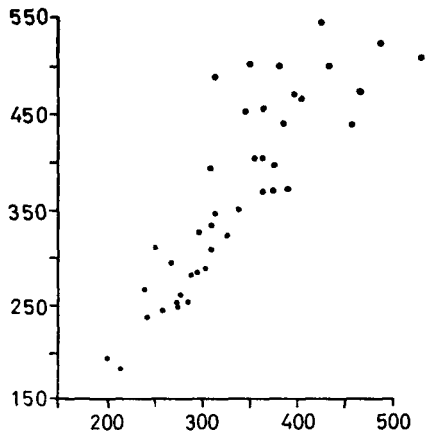

FIG. 1. Plot showing cardiac output obtained using the dye-dilution (ordinate) and the microsphere method (abscissa) simultaneously. Units: ml min^{-1}.

sphere method is shown by comparing the (mean ± s.e.m.; n = 11) blood flow values (ml min^{-1} per 100 g) of identical organs (not included in Tables). For example, the blood flow to left and right kidneys was 418 ± 25 and 451 ± 33, respectively and to left and right ear was 5·3 ± 1·5 and 4·9 ± 0·6, respectively. The blood flow distribution to five different regions in the brain was: left cerebral hemisphere (50 ± 4), right cerebral hemisphere (50 ± 3), cerebellum (53 ± 3), diencephalon (42 ± 3) and brain stem (42 ± 3). Similarly, right leg muscle (6·6 ± 0·6) and skin (11·7 ± 1·5) received approximately twice the blood flow to that of the left leg muscle (2·9 ± 0·5) and skin (5·8 ± 1·2) where the femoral vessels were ligated (see methods).

The distribution of blood flow occurring after methysergide (0·5 mg kg^{-1}) and 5-HT (5 μg kg^{-1} min^{-1}) is shown in Table 2. Methysergide caused a significant increase in the percent of the cardiac output distributed to the heart and ears. The blood flow to these organs and to the brain was significantly increased by methysergide at the expense of that to the lungs which receive microspheres, in addition to bronchial artery, via arteriovenous anastomoses (AVA's) (see Heymann & others, 1977).

Infusion of 5-HT increased the percent of the cardiac output and blood flow distributed to stomach, brain and the heart at the expense of that to lungs, skin and kidneys. Total liver blood flow increased but this was entirely due to the portal (stomach) component. The blood flow to spleen, pancreas, mesentery and liver (hepatic artery) decreased but this was not statistically significantly different from that caused by saline. After the administration of methysergide, the responses to 5-HT were significantly inhibited in lung (AVA + bronchial flow), brain, heart, kidneys, skin, stomach and ears.

DISCUSSION

The measurement of cardiac output by the microsphere method, although slightly underestimated, correlated reasonably well, within the range of values observed, with the dye-dilution method. The values of cardiac output obtained in the present investigation, as was the case with the distribution of cardiac output and blood flows to regional organs, were also similar to those previously reported in anaesthetized rabbits (Neutze & others, 1968; Johnston, 1975). In agreement with most earlier observations (see Garratini & Valzelli, 1965), the present data show that 5-HT increases blood flow to heart and brain mainly at the expense of that to skin, lung and renal vascular bed. Furthermore, we have demonstrated that 5-HT can cause a marked increase in gastric blood flow without affecting that to the small or large intestines. This has recently been reported for histamine (Johnston & Owen, 1975) and it is possible that increased gastric blood flow by 5-HT is mediated by histamine-release (Feldberg & Smith, 1953). Since the cardiac output remained unchanged, 5-HT caused vasodilatation in stomach, heart and brain and vasoconstriction in lungs, skin and kidneys. Administration of methysergide reduced the effects of 5-HT in all regions.

Although the anti-5-HT nature of methysergide has been demonstrated in practically every vascular region, the regional haemodynamic effects of methysergide itself resemble those caused by 5-HT. Both methysergide and 5-HT decreased blood flow to lungs and increased that to heart and brain. As we have found when using higher doses (10 μg kg^{-1} min^{-1}) of 5-HT (Saxena & others, 1978) methysergide also increased blood flow to the ears. The increased blood flow to the brain and the ears is in disagreement with vasoconstriction generally reported with both methysergide and 5-HT in the internal carotid (Saxena, 1972; 1974; Vidrio & Hong, 1976) and the central ear arteries (Garrattini & Valzelli, 1965; Fozard, 1975; Apperley & others, 1976). These studies using the electromagnetic flowmeter or perfusion technique measure the reactivity of the vascular bed without distinguishing between the nutrient (capillary) or non-nutrient (A–V shunts) parts. The use of microspheres of 15 μm diam. predominantly measures nutrient flow, since microspheres of this size can escape through non-nutrient AVA's which are larger than the capillaries (see Hales, 1974; Johnston & Saxena, 1978). Substantial arteriovenous shunt flow has been demonstrated in both ears (Warren & Ledingham, 1974) and intracranial structures (Gillilan & Markesbery, 1963; Rowbotham & Little, 1965; Prosenz, 1972) and 5-HT has been reported to reduce A–V shunting (Rondell, Palmer & Bohr, 1957). Thus it is likely that the reduction by both 5-HT and methysergide in the number of microspheres detected in the lungs was primarily due to a diminution of the number of microspheres escaping entrapment in these tissues through AVA's, ultimately to lodge in the lung capillaries. Indeed, we have recently observed that 5-HT infusion reduced the fraction of microspheres reaching the lungs (normally about 40%) after injection in the common carotid artery (unpublished observations). Therefore, although methysergide can reduce the regional vascular effects of 5-HT, it itself has similar effects on heart, brain and lungs. Though no direct evidence has been offered here, decreased microsphere content in lungs may suggest a reduction of A–V anastomotic flow by the drug. The 'partial agonistic' nature (Saxena, 1974) of methysergide's action on AVA's needs further investigation since sudden opening of AVA's diverting the blood away from the nutrient circulation has been implicated in the pathophysiology of migraine attack (Rowbotham & Little, 1965; Heyck, 1969).

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