Cerebrovascular response to intracarotid injection of serotonin before and after middle cerebral artery occlusion[1]

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SUMMARY The effect of intracarotid injection of serotonin (5-HT) on internal carotid artery flow and oxygen availability (O_2a) of the cerebral cortex was studied in 10 baboons. Vasoconstriction occurred in the vascular bed of the territory supplied by the injected artery. After one middle cerebral artery was occluded the vasoconstrictor effect of 5-HT was more pronounced, particularly in the non-ischaemic hemisphere. The capacity of the cerebral vessels to provide collateral blood flow was reduced in both ischaemic and non-ischaemic areas of brain. As a result of focal cerebral ischaemia, 5-HT may accumulate in the brain and contribute to the progression of infarction.

The functional importance of the biogenic amine serotonin (5-hydroxytryptamine, 5-HT) has been emphasized in several extensive reviews (Woolley, 1962; Garattini and Valzelli, 1965; Mantegazza, 1966). Serotonin is known to be a potent cerebral vasoconstrictor (Raynor, McMurtry, and Pool, 1961; Bohr, Goulet, and Taquini, 1961; Karlsberg, Elliott, and Adams, 1963), and it may be an important aetiological factor in migraine (Lance and Anthony, 1968). Until the present investigation, the possible relationship of 5-HT to the pathophysiology of cerebrovascular disease has been largely concerned with the occurrence of vasospasm after subarachnoid haemorrhage and of vascular changes associated with cerebral trauma (Echlin, 1965; Brawley, Strandness, and Kelly, 1968; Simeone, Ryan, and Cotter, 1968; Arutiunov, Baron, and Majorova, 1970; Wilkins and Odom, 1970). In addition to its vasoconstrictor action, 5-HT depresses the reticular formation, cardiorespiratory centres and the cerebral cortex, and causes cerebral oedema (Marrazzi and Hart, 1955; Gaddum and Vogt, 1956; Bulle, 1957; Costa and Rinaldi, 1958; Monnier, 1960;

Majno and Palade, 1961; Domer and Longo, 1962; Osterholm and Pyenson, 1969).

Changes in the microcirculation of the leptomeningeal vessels have been observed by several investigators after occlusion of a major cerebral artery (Denny-Brown and Meyer, 1957; Meyer and Denny-Brown, 1957; Meyer, 1958). Waltz and Sundt (1967) noted that, after occlusion of the middle cerebral artery in the squirrel monkey, scattered foci of pallor of the cerebral cortex developed which later coalesced. A fluctuating decrease in the calibre of the leptomeningeal vessels was seen in over 90% of their preparations. They mentioned that 5-HT might possibly be released to account for the vasoconstriction and pallor of the cortex.

The hypothesis that altered 5-HT metabolism in ischaemic brain might contribute to the spread of infarction was supported by the work of Bell, Sundt, and Nofzinger (1967) who permanently occluded the middle cerebral artery in the squirrel monkey and applied 5-HT topically. They reported that, in general, the vessels in the ischaemic area were more sensitive to the vasoconstrictive action of 5-HT than the arteries of the non-ischaemic hemisphere. However, in some of the experiments conducted by Bell, the

vessels of the non-ischaemic hemisphere were equally sensitive and, in some instances, more sensitive to the action of 5-HT than the vessels in the ischaemic zones. Waltz and Sundt (1967) also observed some constriction of the arteries and arterioles of the non-ischaemic hemisphere, which was attributed to autoregulation during increases in blood pressure.

Altered vasomotor reactivity in regions of the brain remote from an ischaemic area may be an important consideration in determining the severity of cerebral infarction, since such factors will influence the competence of the collateral circulation. For example, cerebral blood flow has been reported to be depressed in the non-ischaemic cerebral hemisphere in patients with unilateral cerebral infarction, a phenomenon termed diaschisis (Von Monakov, 1914; Høedt-Rasmussen and Skinhøj, 1964; Skinhøj, 1965; Meyer, Shinohara, Kanda, Fukuuchi, Ericsson, and Kok, 1970). It is possible that increased sensitivity to vasoconstrictive stimuli of vessels remote from a localized area of cerebral infarction due to release of 5-HT may be a factor in the aetiology of diaschisis.

The present experiments were designed to measure the effect of intracarotid injection of 5-HT on cerebral collateral blood flow after unilateral cerebral ischaemia produced by temporary occlusion and release of the middle cerebral artery in the baboon.

METHOD

Ten baboons, averaging 6 kg in weight, were anaesthetized with intravenous pentobarbitone, 30 mg/kg, and 0·4 mg atropine was given intramuscularly. Supplemental pentobarbitone was given as required to maintain continuous anaesthesia. Tracheostomy was performed, and the animals were given gallamine triethiodide (Flaxedil); controlled respiration enabled constant levels of expired CO_2 to be maintained (Harvard model 607 respirator). Both femoral arteries and one femoral vein were cannulated with polyethylene catheters in order to measure arterial blood pressure and 5-HT content of the arterial blood and to permit intravenous infusion. A midline cervical incision was made to expose the cervical vessels, and small polythylene catheters for injection of 5-HT were inserted into both internal carotid arteries via the facial artery. The transorbital approach described by Hudgins and

Garcia (1970) was used for occluding the middle cerebral artery. The left middle cerebral artery was occluded in all experiments. The left cerebral hemisphere is referred to in the text as the ischaemic hemisphere and the right the non-ischaemic hemisphere both before as well as after middle cerebral occlusion.

Polarographic electrodes made of platinum wire 2 cm in length and 250 μ in diameter insulated with silicone-phenolic resin (Gagekote no. 4[1]) were used for measuring oxygen availability (O_2a) of the cerebral cortex. The electrodes were placed bilaterally in areas supplied by the middle cerebral artery (designated left parietal and right parietal) and in the area supplied by the anterior cerebral artery bilaterally (designated left frontal and right frontal).

Each oxygen electrode was inserted into the brain tissue to a depth of 3 to 5 mm through a twist-drill hole in the skull. A flattened chlorided silver wire was inserted under the temporalis muscle as an anode. A potential of $-0·6$ V was applied to the platinum wire and a 10 MΩ resistance was added in series with the circuit to stabilize electrical output. Currents generated by the electrodes were amplified by a microvoltmeter (Millivac instrument, model 07C) and recorded with a Grass model 7 polygraph DC amplifier.

The reproducibility of the response of each electrode was assessed by having the animal inhale 5% CO_2 in air for five minutes. The sensitivity of the electrode was considered satisfactory if O_2a of the brain tissue increased more than 20%.

Two gated sine wave electromagnetic flowmeter probes were placed around both common carotid arteries. Both external carotid arteries were ligated, and flow in the common carotid artery was used for measuring internal carotid artery flow. Pulsatile flow was recorded on the Grass polygraph.

The 5-HT content of whole blood was measured using an autoanalytical procedure developed in this laboratory (Welch and Meyer, 1972). Cerebral venous blood and arterial blood were sampled from catheters placed in the sagittal sinus and femoral artery. Blood samples were transported to two autoanalytical systems by means of a proportioning pump (Technicon[2]) for measurement of whole blood 5-HT levels during experiments in which 5-HT was injected into the carotid artery.

End-tidal CO_2 was measured with an infra-red gas analyser (Beckman, Spinco model 3 LB-1). Arterial blood pressure, electroencephalogram (EEG), and electrocardiogram (ECG) were continuously monitored. Body temperature was maintained between 35° and 37° C with a heating pad.

[1] Wm. T. Bean, Detroit, Michigan.
[2] Technicon Instruments Corporation, 511 Benedict Avenue, Terrytown, New York.

The experiment was conducted according to the following protocol. Steady state values were obtained before occlusion. A solution of 5-HT-hydrogen oxalate (Calbiochem[3]) was infused at a rate of 20 μg/kg body wt/min into the internal carotid artery of one hemisphere with a Harvard infusion pump. Equal quantities of 5-HT were used for each injection. Brain tissue O_2a and internal carotid artery flow were measured for 30 minutes after injection or until return to approximately steady state levels. 5-HT was then injected into the opposite internal carotid artery and observation made over a similar time course. In some experiments, two 5-HT injections were made into each artery before occlusion of the middle cerebral artery.

Internal carotid artery flow and O_2a were measured for a 30 minute period both during and after clamping of the middle cerebral artery. After release of occlusion, intracarotid injection of 5-HT into both hemispheres was repeated in a manner similar to that before occlusion. A total of 46 injections of 5-HT was made in 10 baboons.

O_2a of brain tissue was expressed as percentage of the steady state value before each procedure. Internal carotid artery flow, calculated in ml./min, also was

[3] Calbiochem, P.O. Box 54282, Los Angeles, California.

expressed as a percentage of the steady state value before each procedure to permit convenient comparison with O_2a values. Whole blood 5-HT content was measured in arterial and cerebral venous blood, and the cerebral arteriovenous (A–V) differences expressed in nanograms (ng)/ml of whole blood. The results were analysed using Student's t test. The percentage changes cited for both flow and cortical PO_2 changes were regarded as statistically significant at or better than a 95% confidence limit.

At the end of the experiment the brain was removed to verify the site where the clip on the middle cerebral artery was placed and to confirm that no trauma or haematoma had occurred.

RESULTS

BLOOD PRESSURE   Before occlusion of the middle cerebral artery in 26 experiments, mean arterial blood pressure (MABP) did not change significantly during the period of 5-HT injection or for 25 minutes after the injection was terminated. MABP during the steady state was 127 ± 20 mmHg. No significant change was observed after injection in 20 instances during the post-ischae-

TABLE 1A

EFFECT OF INTRACAROTID 5-HT INJECTION ON IPSILATERAL INTERNAL CAROTID ARTERY FLOW* BEFORE LEFT MIDDLE CEREBRAL ARTERY OCCLUSION TO SHOW SIGNIFICANT REDUCTION IN BLOOD FLOW

				Time (min)			
	SS	5	10	15	20	25	30
Total injections							
Mean	100	52	50	56	62	67	66
SD		23	26	28	31	37	51
N	26	26	26	26	25	22	14
P value		<0·001	<0·001	<0·001	<0·001	<0·001	<0·01

SS = steady state.

TABLE 1B

REPRODUCIBILITY OF EFFECT OF REPEATED INTRACAROTID SEROTONIN INJECTIONS ON IPSILATERAL INTERNAL CAROTID FLOW* BEFORE MIDDLE CEREBRAL ARTERY OCCLUSION TO SHOW NO SIGNIFICANT DIFFERENCE BETWEEN INJECTIONS

	Injection											
	1	2	1	2	1	2	1	2	1	2	1	2
First and second injections												
Mean	62	66	67	54	70	65	71	67	71	82	81	—
SD	37	23	31	31	33	33	32	30	42	32	32	—
N-2	10		10		10		10		9			
P value	NS		NS		NS		NS		NS		NS	

* Expressed as percentage change from steady state value.　　SD = standard deviation.　　N = number of values　　SS = steady state.
NS = not significant.

TABLE 2

EFFECT OF INTRACAROTID INJECTION OF 5-HT ON IPSILATERAL CORTICAL O_2a* BEFORE LEFT MIDDLE CEREBRAL ARTERY OCCLUSION

	Time interval after injection of serotonin (min)						
	SS	*5*	*10*	*15*	*20*	*25*	*30*
	(%)	(%)	(%)	(%)	(%)	(%)	(%)
Total injections							
Mean	100	92	95	96	96	100	103
SD		16	17	21	23	25	29
N	44	44	44	44	44	42	26
P value		<0·001	NS	NS	NS	NS	NS

* Percentage change from steady state value.   SD = standard devation.   N = number of values.   SS = steady state.
NS = not significant.

mic period. MABP during the new steady state (after ischaemia) was 121 ± 28 mmHg. There was no significant difference between pre- and post-ischaemic MABP values.

ALVEOLAR CO_2 The mean end-tidal CO_2 was $3·8 \pm 0·6\%$ for the 46 injections of 5-HT. No significant change took place before or after any of the experimental procedures.

ELECTROENCEPHALOGRAPHY   Generalized slow wave activity, more pronounced in the injected hemisphere, occurred after almost all unilateral intracarotid injections of 5-HT. Activity usually returned to normal within 10 to 15 minutes. The induced focal cerebral ischaemia did not exaggerate this effect of intracarotid injection of 5-HT on the EEG.

HAEMODYNAMIC CHANGE DURING AND IMMEDIATELY AFTER MIDDLE CEREBRAL ARTERY OCCLUSION   Mean flow in the ipsilateral internal carotid artery decreased significantly during the 30 minute period of middle cerebral occlusion then increased significantly above pre-occlusion values immediately after the vessel was unclamped. Internal carotid flow of the non-ischaemic hemisphere did not alter.

O_2a in the territory of the occluded middle cerebral artery decreased promptly and significantly. In a few instances after the clamp was released hyperoxia was noted in areas of previously ischaemic cortex. However, the mean O_2a in the ischaemic parietal cortex remained significantly reduced (at 85% of the steady state

level) 25 minutes after release of occlusion $(P<0·05)$. Significant increase in mean O_2a of the left frontal region during parietal ischaemia presumably reflected the development of collateral flow. Cortical O_2a did not alter in the non-ischaemic hemisphere.

EFFECT OF 5-HT BEFORE CEREBRAL ISCHAEMIA   Injection of 5-HT (20 μg/kg/min) into the internal carotid artery significantly decreased ipsilateral internal carotid flow to both hemispheres (Table 1A). There was no significant difference between the two vessels in the amount of decrease. Flow always increased in the carotid vessel contralateral to that injected, presumably because it acts as a collateral channel. However, such collateral flow was significantly less in the internal carotid artery of the hemisphere which had been prepared for the middle cerebral artery occlusion $(P<0·05)$.

The reproducibility of the response to repeated injections into the same artery (Table 1B) indicates that neither tachyphylaxis nor sensitization of the carotid vessels developed.

Changes in cortical O_2a were similar to those of carotid flow. O_2a of the ipsilateral, frontal, and parietal cortex decreased significantly and consistently with repeated injection of 5-HT (Table 2). In accordance with observed increases in carotid flow, O_2a of the parietal cortices contralateral to the injected hemispheres increased towards the end of the period of observation (see Figs 3B and 4B), although there was an initial decrease in O_2a of the non-ischaemic parietal cortex, probably due to movement of
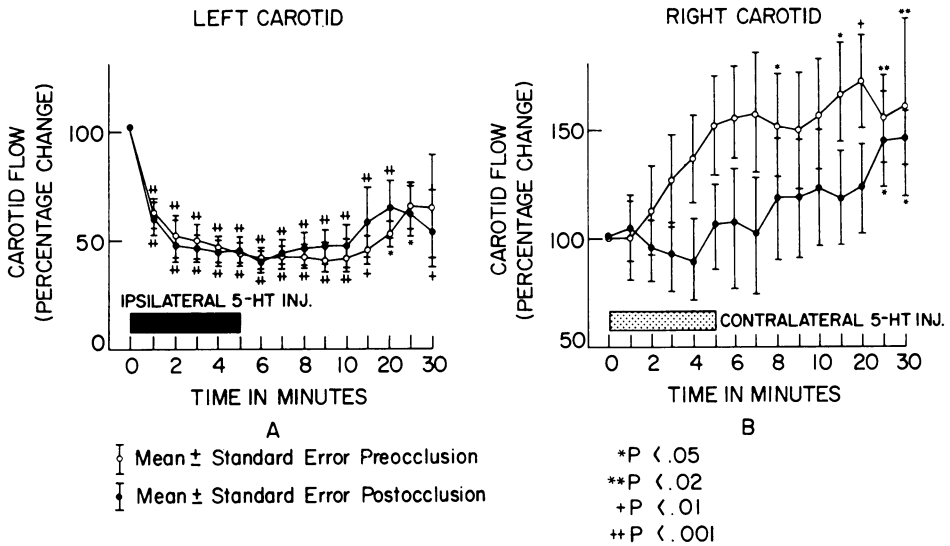
FIG. 1. *Comparison of percentage changes in ipsilateral and contralateral internal carotid artery flow before and after ischaemia when 5-HT was injected to the left ischaemic hemisphere. The P values for statistical significance of changes from steady state values before ischaemia (○) are indicated by symbols above the lines and for changes from steady state values after ischaemia (●) by symbols below the lines.*

5-HT to the non-injected hemisphere via the circle of Willis (see Fig. 3B).

EFFECT OF 5-HT AFTER CEREBRAL ISCHAEMIA When 5-HT was injected to the ischaemic hemisphere ipsilateral carotid artery flow decreased significantly and, although the decrease was more prolonged, the values noted were not significantly different from those obtained before ischaemia. On these occasions collateral flow in the contralateral internal carotid artery was reduced and delayed (Fig. 1B). Changes identical with those of carotid flow were observed in O_2a of the ischaemic and the contralateral non-ischaemic parietal cortex, except that there was the same initial decrease in contralateral parietal O_2a that was observed before ischaemia (see Fig. 3A and B). However, frontal cortical O_2a of the ischaemic hemisphere did decrease more rapidly and to a greater degree than before ischaemia upon ipsilateral 5-HT injection (see Fig. 5).

When 5-HT was injected to the non-ischaemic hemisphere ipsilateral carotid flow decreased to a significantly greater degree compared with before ischaemia. On these occasions significant collateral flow was not observed in the contralateral carotid artery (Fig. 2B). There was also an earlier greater and more sustained reduction noted in ipsilateral parietal O_2a compared with before ischaemia (Fig. 4A). Again on these occasions contralateral parietal O_2a of the ischaemic cortex was also significantly reduced, unlike before ischaemia when hyperoxia was observed (Fig. 4B).

MEASUREMENT OF 5-HT IN FEMORAL ARTERIAL AND CEREBRAL VENOUS BLOOD Concentrations of 5-HT in the cerebral venous (V) and femoral arterial (A) blood were measured after eight injections before ischaemia and eight injections after ischaemia in order to determine the 'washout' of 5-HT from the brain. The A–V differences for 5-HT are shown in Table 3. A negative A–V difference was apparent during the so-called steady state both before and after ischaemia. The mean negative A–V difference in the steady state is due to the fact that the 5-HT levels were not determined in a true steady state
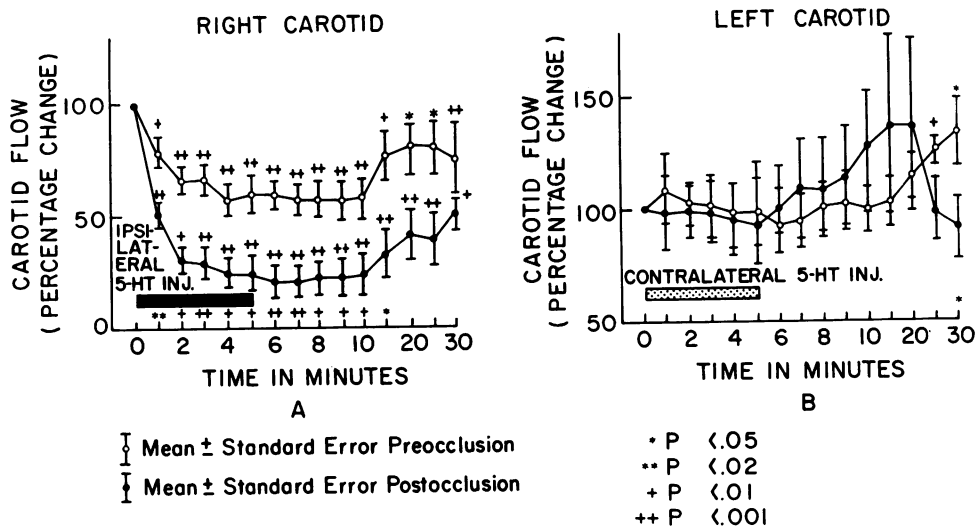
FIG. 2. *Comparison of percentage change in ipsilateral and contralateral internal carotid artery flow before and after ischaemia when 5-HT was injected to the right non-ischaemic hemisphere. The P values for statistical significance of changes from steady state values before ischaemia (○) and after ischaemia (●) are indicated by symbols above the lines. Additional P values for the statistical significance of difference between internal carotid artery flow values before and after ischaemia at each minute of time are indicated by symbols below the marker for 5-HT injection.*
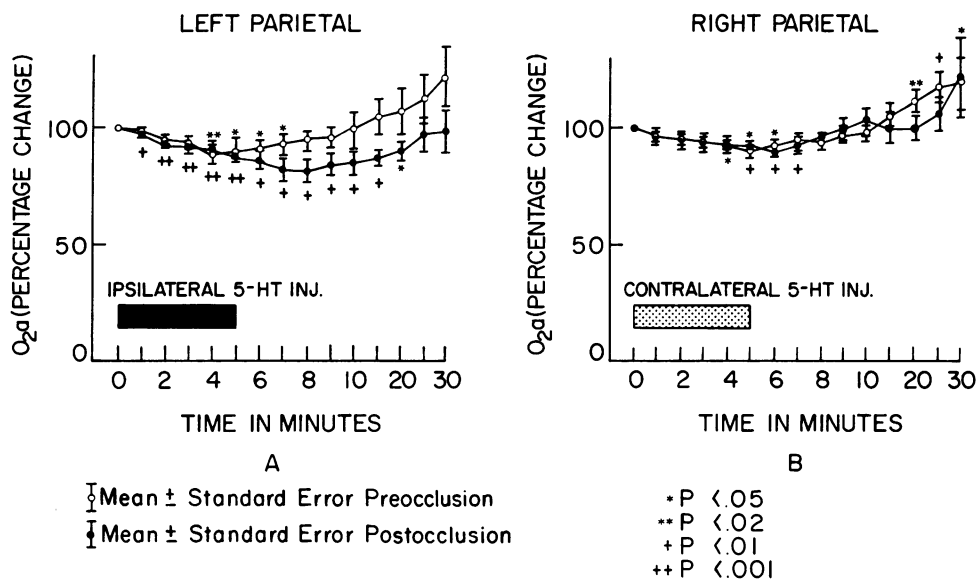

FIG. 3. *Comparison of percentage changes in ipsilateral and contralateral parietal O_2a before and after ischaemia when 5-HT was injected to the left ischaemic hemisphere. The P values for statistical significance of changes from steady state values before ischaemia (○) are indicated by symbols above the lines and for changes from steady state values after ischaemia (●) by symbols below the lines.*
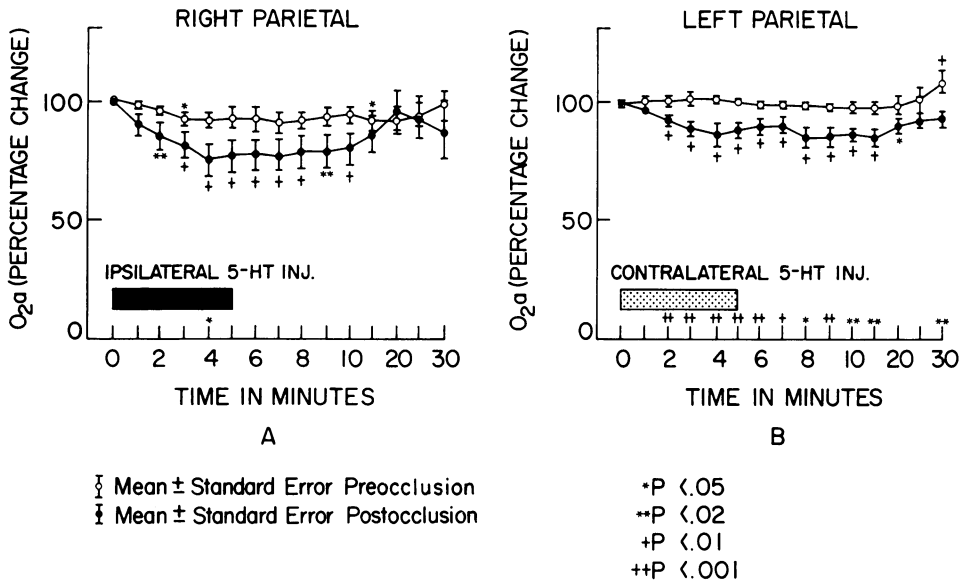
FIG. 4. *Comparison of percentage change in ipsilateral and contralateral parietal O_2a before and after ischaemia when 5-HT was injected to the right non-ischaemic hemisphere. The P values for statistical significance of changes from steady state values before ischaemia ($\bigcirc$) are indicated by symbols above the lines and changes from steady state values after ischaemia ($\bullet$) by symbols below the lines. Additional P values for the statistical significance of difference between O_2a values before and after ischaemia at each minute of time are indicated by symbols below the marker for 5-HT injection.*

TABLE 3

MEASUREMENT OF FEMORAL AND CEREBRAL A–V DIFFERENCES FOR WHOLE BLOOD SEROTONIN* AFTER INTRACAROTID INJECTION OF 5-HT (20 μG/KG/MIN) FOR FIVE MINUTES COMPARED WITH STEADY STATE VALUES IN EXPERIMENTS BEFORE AND AFTER LEFT MIDDLE CEREBRAL ARTERY OCCLUSION

				Minutes†				
	SS	*6*	*8*	*10*	*15*	*20*	*25*	*30*
Preocclusion								
Mean	− 72	− 559	− 685	− 705	− 206	− 196	− 211	− 154
SD	33	944	804	828	106	94	102	89
N	8	8	8	8	8	8	8	8
P value	—	NS	< 0·05	< 0·05	< 0·01	< 0·01	< 0·01	< 0·05
Postocclusion								
Mean	− 102	− 742	− 636	− 403	− 277	− 232	− 237	− 179
SD	71	1,050	848	634	270	199	207	261
N	8	8	8	8	8	8	8	8
P value	—	NS	NS	NS	NS	NS	NS	NS

* Expressed in nanograms (ng)/ml. whole blood.   † Time in minutes from start of injections.
SD = standard deviation.   SS = steady state.   N = number of values.   NS = not significant.
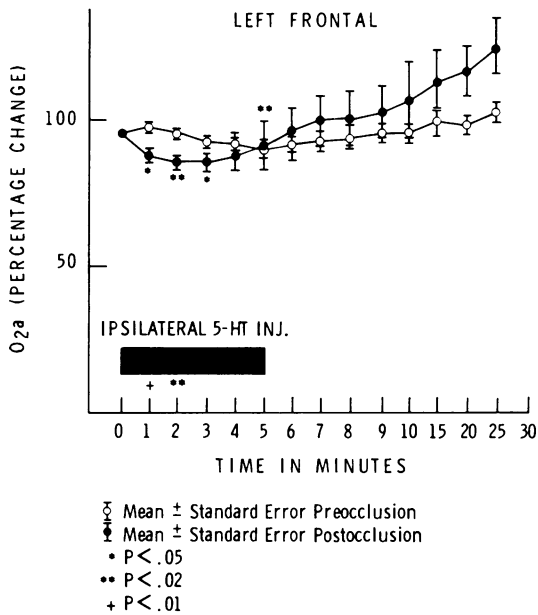
FIG. 5. *Comparison of percentage change in ipsilateral O_2a of the left frontal cortex of the ischaemic hemisphere before and after ischaemia when 5-HT was injected to the ischaemic cortex. The P values for statistical significance of changes from steady state values before ischaemia ($\bigcirc$) are indicated by symbols above the lines and for change from steady state values after ischaemia ($\bullet$) by symbols below the lines. Additional P values for the statistical significance of difference of O_2a values before and after ischaemia at each minute of time are indicated by symbols below the marker for 5-HT injection.*

but in experiments after the first injection of 5-HT already had been made.

Levels of 5-HT were calculated from 1 to 25 minutes after discontinuation of 5-HT injection. Before ischaemia the A–V difference or cerebral wash-out was statistically significant. This was not so after ischaemia.

DISCUSSION

PHYSIOLOGICAL EFFECT OF INTRACAROTID 5-HT INJECTION   Injection of 5-HT, 20 μg/kg/min, into the internal carotid artery of the baboon produced a significant decrease in internal carotid artery flow. Inasmuch as no change took place in MABP or alveolar CO_2, it is concluded

that intra-arterial injection of 5-HT causes increased cerebrovascular resistance, confirming similar observations by other investigators (Karlsberg *et al.*, 1963; Grimson, Robinson, and Danford, Tindall, and Greenfield, 1969; Deshmukh and Harper, 1971). Constriction of the internal carotid artery after 5-HT injection was noted on arteriography in two of these studies (Grimson *et al.*, 1969; Deshmukh and Harper, 1971). Mchedlishvili (1969) demonstrated a direct vasoconstrictor effect by 5-HT on the isolated carotid artery in the dog in the absence of an autonomic nerve supply. Topical application of 5-HT has also been reported to cause constriction of the pial arteries (Raynor *et al.*, 1961). The studies of Grimson *et al.* (1969) indicate that total intracranial cerebrovascular resistance may be increased by unilateral intracarotid injection of 5-HT.

In our preparations, when 5-HT was injected into one internal carotid artery, flow in the opposite vessel increased, presumably due to its action as a collateral vessel to replenish the territory supplied by the injected vessel. However, this capacity to provide collateral flow was less marked in the carotid artery of the hemisphere prepared for middle cerebral artery occlusion, possibly due to the operative preparation itself which necessitates enucleation of the orbital contents and ligation of the ophthalmic artery. The opposite ophthalmic artery is left intact and may serve as a collateral vessel despite ligature of the external carotid artery, since extracranial tissues were not surgically removed. This may account for the observed differences in collateral flow. An alternative explanation might be that 5-HT when injected to the opposite hemisphere passes to the extracranial vasculature via the circle of Willis and patent right ophthalmic artery. Should 5-HT increase extracranial blood flow as suggested by Deshmukh and Harper (1971), this could also account for the increased collateral flow in the right carotid artery over the left. However, this mechanism seems less important since cortical flow increased with carotid flow (Fig. 3B). In the same way, since regional blood flow changes measured with the brain implanted oxygen electrodes corresponded well with the carotid flow changes, we do not believe extracranial contamination was a practical problem in our studies.

The validity of polarographic electrodes used in our experiments to measure O_2a of brain tissue as an indication of local circulatory changes has been discussed in previous papers (Meyer, Fang, and Denny-Brown, 1954; Clark, Misrahy, and Fox, 1958; Halsey and Clark, 1970). A decrease in cortical O_2a measured with the electrodes in the hemisphere on the injected side was noted after intracarotid injection of 5-HT. The percentage reduction in O_2a from steady state levels was less than expected from the percentage reduction of internal carotid artery flow, possibly due to the depressant effect of 5-HT at the levels injected on oxygen consumption (such an effect would tend to obscure the fall in cerebral O_2a caused by decreased regional cerebral blood flow) (Halsey and Clark, 1970) or 5-HT may have a selectively greater vasoconstrictor effect on the larger branches of the internal carotid distribution (Lende, 1960).

Deshmukh and Harper (1971) simultaneously measured mean regional cerebral blood flow determined from [133]Xenon clearance curves and internal carotid artery flow with electromagnetic flowmeters. In a small number of experiments, mean regional cerebral blood flow did not change significantly after intracarotid 5-HT injection despite a decrease in the internal carotid flow associated with marked constriction of the internal carotid artery. The bolus method requires at least 10 minutes to measure cerebral blood flow, so that some of the rapid changes measured here may not have been measured for technical reasons. The reduction of cortical O_2a levels in our series of experiments suggest that vasoconstriction occurs in the vascular bed of the territory supplied by the artery injected with 5-HT in agreement with other studies (Grimson *et al.*, 1969).

EFFECT OF 5-HT INJECTION TO ISCHAEMIC HEMISPHERE   There was no convincing difference in the reduction of carotid flow after ipsilateral 5-HT injection before and after ischaemia. Similar observations were made by measuring parietal O_2a of the ischaemic cortex, although for both measurements the reduction in flow from steady state values was prolonged. Bell *et al.* (1967) observed significant increases in the vasoconstrictor response of the cerebral microvasculature to topically applied 5-HT in areas of brain rendered ischaemic by permanent occlusion of the middle cerebral artery. They concluded that cerebral ischaemia sensitized these vessels to the vasoconstrictor effects of 5-HT. In the present experiments, O_2a in the ischaemic cortex returned to only 85% of the steady state value after the left middle cerebral artery was unclamped, suggesting reduced regional blood flow. Yet when 5-HT was injected to the ischaemic hemisphere after middle cerebral occlusion the percentage reduction in ipsilateral parietal O_2a, apart from being prolonged, was identical with preocclusion values. This suggests the possibility of increased vasoconstrictor effect of 5-HT on vessels in the ischaemic region. However, convincing alteration in response of the vessels in the ischaemic region to 5-HT is shown when 5-HT is injected into the non-ischaemic hemisphere (Fig. 4B). Either movement of 5-HT to the ischaemic hemisphere via the circle of Willis, or recirculating 5-HT, reduced O_2a of the ischaemic cortex by a vasoconstrictor effect which was not demonstrated before occlusion. Although not absolute, the results of this study tend to support the report of Bell *et al.* (1967) that cerebral ischaemia increases the response of the cerebral microvasculature in the ischaemic area to the vasoconstrictor action of 5-HT.

EFFECT OF 5-HT INJECTION TO NONISCHAEMIC HEMISPHERE   The reduction of internal carotid blood flow and cortical O_2a of the nonischaemic hemisphere after injecting 5-HT was more pronounced after ischaemia. There was neither evidence of tachyphylaxis nor sensitization of the cerebral vessels to 5-HT (Table 2). Since blood pressure and end-tidal CO_2 content were constant, these observations suggest an increased vasoconstrictor effect of 5-HT on the cerebral vessels in the non-ischaemic hemisphere, presumably resulting in some way from ischaemia of the opposite hemisphere. Since the right hemisphere was neither ischaemic nor anoxic, as judged by carotid flow and cortical O_2a levels, four possible explanations are offered to account for this apparent increased vasoconstrictor effect of 5-HT on the cerebral vessels of the intact hemisphere:

1. The greater reduction of flow in the ischaemic hemisphere produced by 5-HT injection after middle cerebral artery occlusion may

be due to the decreased capacitance of the vessels in the ischaemic left parietal zone to provide collateral flow, as seen in Figs 2B and 4B.

2. A central and neurogenically mediated alteration in vasomotor tone and reactivity to humoral elements secondary to localized cerebral ischaemia.

3. Ischaemia of one hemisphere causes increased levels of free 5-HT in the unaffected areas of brain, cerebrospinal fluid (CSF), or circulating blood which enhance the vasoconstrictor effect of injected free 5-HT.

4. Alteration in brain tissue, CSF, or circulating blood levels of some other substance or substances as a result of brain ischaemia which augments the vasoconstrictor properties of 5-HT.

The extraordinarily rapid and much greater vasoconstrictor effect of injected 5-HT seen in this instance after induced brain ischaemia serves to dismiss the first hypothesis, since mechanical reduction of collateral flow in the ischaemic hemisphere would be expected to have a more delayed effect. Strong support is given to the other three possibilities by the observation that 5-HT injected to the ischaemic hemisphere before middle cerebral artery occlusion causes increased collateral flow in the non-ischaemic hemisphere which was not as increased or sustained when similar injections of 5-HT were made after ischaemia (see Figs 1B and 3B). In addition, there was an increased vasoconstrictor effect of 5-HT on collateral vessels in the ischaemic hemisphere itself (Fig. 5). This loss of collateral capacitance could best be explained by the vasoactivity of elevated 5-HT levels, or increases in levels of some other vasoconstrictive factor in brain, CSF, or blood alone, or in combination with a central neurogenically mediated effect as suggested. The subject of central neurogenic control of cerebral vasoactivity is ill-understood and controversial (Rosenblum, 1971). However, alteration of some such control cannot be excluded as a partial explanation of some of the findings of this study.

Collateral flow is initially a passive phenomenon governed by intravascular pressure differences throughout the circulation (Symon, Ishikawa, and Meyer, 1963). Therefore, factors that will impair collateral flow must be: obstruction of the vascular lumen—for example, by endothelial swelling or aggregation of blood

elements; compression of vessels from without by oedema of perivascular glial cells; or increased tone of the vessel wall itself. In our experiments, some of these conditions were already in evidence after removal of occlusion, since regional blood flow in the ischaemic area returned to only 85% of its preocclusion value. Bell *et al.* (1967) found increased reactivity of ischaemic vessels to the vasoconstrictor action of topically applied 5-HT. Supportive evidence from their study, particularly the altered reactivity of ischaemic vessels (Fig. 4B), serves to implicate 5-HT as one factor responsible for increased tone of the ischaemic vessels. Since 5-HT contributes to impairment of collateral flow, it seems warranted to consider this substance further in the aetiology of poor perfusion (Ames, Wright, Kowada, Thurston, and Majno, 1968) after cerebral infarction.

It is well known that cerebral vessels in non-ischaemic areas of brain can act as collateral channels for blood flow to the territory supplied by an occluded vessel. What has emerged from this study is that the capacity of these same cerebral vessels to provide collateral flow may be impaired by ischaemia.

Release of 5-HT from the platelets in cerebral blood and possible accumulation of 5-HT in brain tissue after ischaemic anoxia due to damage to the blood-brain barrier, have been demonstrated already in another series of experiments in this laboratory (Welch, Meyer, Teraura, Hashi, and Shinmaru, 1972). Evidence to support the latter was obtained in the present experiments from measurement of femoral arterial and cerebral venous differences for 5-HT after injection into the carotid artery. Before middle cerebral artery occlusion, a significant cerebral wash-out of 5-HT was measured (Table 5). After occlusion of the middle cerebral artery this significant wash-out was no longer present, indicating either increased movement of 5-HT from blood into brain caused by ischaemic damage to the blood–brain barrier or else delayed wash-out of 5-HT from the cerebral vasculature due to slowing of capillary and venous blood.

Free 5-HT accumulated in brain tissue from the blood after cerebral ischaemia could fully preempt receptor sites. The monamine oxidases present in brain, which are reduced by ischaemic

anoxia (Păuşescu, Lugojan, and Păuşescu, 1970), could be insufficient to metabolize the excess 5-HT (Brodie and Reid, 1968). Brain tissue levels of 5-HT elevated in this way move into CSF to produce diffuse effects on brain function. Studies of 5-HT levels in the blood of patients with cerebrovascular disease have been contradictory, in that increased, normal, and decreased levels of 5-HT were found (Krieger, Kolodny, and Warner, 1964; Wilkins, Silver, and Odom, 1966; Berzin, Auna, and Brezhinskiy, 1969). Another explanation for increased 5-HT in nonischaemic cerebral cortex, brain-stem, and CSF after cerebral infarction may be that compensatory metabolism of the serotonergic system increases as a result of 5-HT release into areas of brain damage. Osterholm and Ryenson (1969) reported this in cats after experimentally induced cerebral trauma.

The role of altered 5-HT metabolism in the pathophysiology of cerebral infarction is supported by elevated levels of 5-HT found in the CSF of patients with thromboembolic and haemorrhagic cerebrovascular disease (Southern and Christoff, 1962; Misra, Singh, and Bhargava, 1967; Berzin *et al.*, 1969). Stoica and colleagues (Stoica and Nash, 1966; Stoica, Păuşescu, and Trandafirescu, 1968) demonstrated increased reactivity of the cerebral vessels to cold stress in patients with cerebrovascular disease and related this observation to changes in 5-HT levels in the blood of these patients. Their findings seem particularly pertinent to our present study. Berzin *et al.* (1969) proceeded even further with this concept, relating the severity of the clinical condition after various types of stroke to increases in 5-HT blood levels in patients with cerebrovascular disease.

That altered 5-HT metabolism may be implicated in the aetiology of diaschisis can be only speculative. It does not seem unreasonable, however, to hypothesize that increased sensitivity to vasoconstrictive stimuli of the vessels in areas remote from localized cerebral ischaemia may be partially responsible for some reduction of cerebral blood flow to areas of brain not involved in the ischaemic process.

The present study indicates that altered 5-HT metabolism may affect the adequacy of the collateral circulation and may be an active factor in progressive reduction of cerebral blood flow in both the ischaemic and non-ischaemic cerebral hemispheres of the brain. The role that 5-HT and other neurotransmitters may play in the genesis of cerebral oedema, neuronal depression, and diaschisis is being investigated further.

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