J. Physiol. (1977), **271**, *pp.* 735–746
With 4 text-figures
Printed in Great Britain

EFFECTS OF 5-HYDROXYTRYPTAMINE ON PIAL ARTERIOLAR CALIBRE IN ANAESTHETIZED CATS

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(*Received 1 February 1977*)

SUMMARY

1. The responses of individual pial arterioles and small arteries to perivascular injections of 5-hydroxytryptamine were studied in anaesthetized cats.

2. Pial arteriolar calibre was measured by a television image-splitting technique.

3. At normotension, the average response of pial vessels was dilatatory at injected 5-hydroxytryptamine concentrations between 10^{-10} and 10^{-4} M.

4. These pial vascular changes were, however, dependent upon the resting vessel calibre: those arterioles $< 70\ \mu m$ dilated universally, whereas small arteries $\geqslant 200\ \mu m$ in resting diameter tended to constrict in response to 5-hydroxytryptamine.

5. The 5-hydroxytryptamine-induced dilatation of pial arterioles $< 70\ \mu m$ in resting calibre was directly dependent on mean arterial pressure, although no such relationship was observed with the larger cerebral vessels.

6. It is concluded that the specific cerebrovascular actions of 5-hydroxytryptamine are largely tone-dependent. The greater the resting tone of a pial arteriole, then the greater will be the tendency of that vessel to dilate in response to 5-hydroxytryptamine, and vice versa.

7. The direct vascular actions of 5-hydroxytryptamine are in contradistinction to the observed changes in cerebral tissue perfusion and it would therefore appear that the effects of this amine on cerebral metabolism and neuronal activity outweigh its cerebrovascular actions under physiological conditions.

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INTRODUCTION

In the preceding paper it was shown that the intracarotid administration of 5-hydroxytryptamine could decrease cerebral tissue perfusion, but that this effect could not be separated from concomitant changes in cerebral metabolism and electrocortical activity. Furthermore, the concentration of 5-hydroxytryptamine that was infused (0·1 μg/kg.min) was only effective following transient disruption of the blood–brain barrier (Harper & MacKenzie, 1977).

Accordingly, it was decided to investigate the direct cerebrovascular actions of 5-hydroxytryptamine by examining the effects of topical 5-hydroxytryptamine on pial arteriolar calibre *in vivo*. By injecting small amounts of 5-hydroxytryptamine into the subarachnoid space surrounding a single pial arteriole it was hoped to avoid two factors which complicated our previous investigation. These were, first, the blood–brain barrier mechanisms to monoamines and, secondly, the activation of changes in either cerebral metabolism or neuronal function. Transient changes in pial arteriolar calibre were measured by a television image-splitting technique in anaesthetized cats.

METHODS

General. Anaesthesia was induced in fourteen cats (2·0–4·5 kg body weight) by the rapidly metabolized combination of alphaxolone (6·75 mg/kg) and alphadolone acetate (2·25 mg/kg), administered I.V. The cats were intubated with a cuffed endotracheal tube and cannulae were inserted into the abdominal aorta and inferior vena cava, via the femoral vessels. Anaesthesia was maintained with α-chloralose (6 ml./kg of a 10% solution, I.V.) and additional α-chloralose was given when needed to abolish the corneal reflex.

Positive pressure ventilation with 100% oxygen was used throughout and either the rate or the tidal volume of the respirator was adjusted to maintain normocapnia ($P_{a,CO_2} \approx 32$ mmHg; range 29–35 mmHg). In a few animals, gallamine (3 mg/kg, I.V.) was administered in order to control ventilation. End-tidal carbon dioxide tension was recorded continuously, and arterial P_{CO_2}, P_{O_2} and pH were measured frequently. Mean arterial pressure was recorded continuously through the catheter in the abdominal aorta. The cats received an I.V. infusion of Hartmann's solution (0·1 ml./min) throughout the experiment. The composition of the Hartmann's solution was: Na^+ 131 mM, K^+ 5 mM, Ca^{2+} 2 mM, HCO_3^- 29 mM and Cl^- 111 mM. Body temperature was maintained at 37 °C by a heating blanket.

Measurement of pial arteriolar calibre. The cats were placed in a head-holder, the scalp incised along the mid line and the cut edges of the scalp were reflected to form a bath for mineral oil, later applied. A dental drill was used to perform a 2·0 × 1·0 cm craniotomy over one parietal cortex, the dental drill being cooled by a jet of saline. The underlying dura mater was removed and the cut dural margins were sealed by bipolar diathermy. Heated mineral oil continuously irrigated the exposed cortex so as to maintain the brain surface temperature at 38 °C by altering either the flow rate or the temperature of the oil.

Pial arteriolar calibre was measured by a television image-splitting technique

(Baez, 1966). The vessels were observed with a Bausch and Lomb stereomicroscope at magnifications of either × 40 or × 70 on the microscope. To measure vessel diameter, the image was passed through an image-splitting eyepiece (Vickers) and the split image was then viewed through the use of a light sensitive television camera (Grundig FA 70) and a video monitor. The degree of image-splitting is controlled by the shearing screw of the eyepiece which was connected to a sensitive potentiometer and, in turn, a direct-reading pen recorder. Thus frequent measurements of shear (which is directly proportional to vessel calibre) could be obtained. The system was calibrated against filaments of known diameter before each experiment so that the absolute diameter of each vessel could be calculated in μm. The surface of the brain was illuminated by a cold light source (Schott fibre optic system).

We have shown previously that the coefficient of variation for repeated measurements of pial arteriolar diameter is 1%, using this system and under conditions of steady arterial pressure and blood gas tensions (MacKenzie, Strandgaard, Graham, Jones, Harper & Farrar, 1976).

Perivascular injection of solutions. 5-Hydroxytryptamine creatinine phosphate was dissolved in a mock cerebrospinal solution of the following composition: Na^+ 156 mM, K^+ 3 mM, Ca^{2+} 1·5 mM, Cl^- 151 mM and HCO_3^- 11 mM. A gas mixture containing 5% carbon dioxide and 95% oxygen was bubbled through the solution which was stored under oil and used within an hour of preparation. The final pH of the solution was 7·15 and the osmolarity of the mock cerebrospinal fluid was 300 m-osmole/l. The solutions were drawn up into sharpened glass micropipettes, again the solutions being sealed on top with a layer of mineral oil, while the tips were stored under oil until immediately prior to use.

The micropipettes were positioned, through the use of a micromanipulator, into the subarachnoid space which invested one particular pial arteriole or artery. Each pial arteriole is studied once only or, more precisely, the diameter of the pial arteriole at the point of injection is examined on a single occasion. By applying pressure to a hydraulic syringe which was attached to the micropipette, approximately 2 μl. could be injected into the perivascular space at one time. The pial vessel calibre was measured for at least 2 min following the injection and the maximum response was compared to the preinjection, base line diameter. On a number of occasions 5-hydroxytryptamine oxalate was used in place of the creatinine phosphate salt but, as the results did not significantly differ at any particular molarity of the base, the results presented are computed from those observed from 5-hydroxytryptamine creatinine phosphate alone. The effect of the mock cerebrospinal fluid solution, by itself, was examined additionally.

Both the method of measuring pial arteriolar calibre and the perivascular application of drug solutions are based essentially on those described by Wahl, Kuschinsky, Bosse & Thurau (1973).

Induction of haemorrhagic hypotension. In five of the cats, mean arterial pressure was lowered by bleeding them into a reservoir, which was heparinized and maintained at 37 °C, from a second aortic catheter. This reservoir was connected to a sphygmomanometer, thus allowing the system to be held at any desired pressure. The cats were heparinized ($\sim$ 300 i.u./kg) at this stage of the experiment. Stepwise reductions in mean arterial pressure were obtained by the intermittent withdrawal of blood, such that pressure was reduced by 10–15 mmHg on each occasion. Once the desired mean arterial pressure had been obtained, the pressure was held constant for 5–10 min before the pial vascular effects of 5-hydroxytryptamine were examined.

RESULTS

The perivascular application of 5-hydroxytryptamine in concentrations between 10^{-10} and 10^{-3} M, resulted in an over-all dilatation of the pial arterioles and arteries, as is shown in Table 1. Nevertheless, on a number of occasions a constriction, rather than dilatation, of these vessels was observed following the administration of 5-hydroxytryptamine and this factor accounts for the large standard error of the mean in the tabulated results. This standard error cannot be ascribed to the technique alone because the perivascular administration of the mock cerebrospinal solution to eleven vessels resulted in a $0 \pm 1{\cdot}4\%$ change from resting vessel diameter (mean $\pm$ 1 S.E. of the mean).

TABLE 1. Effects of the perivascular application of 5-hydroxytryptamine on pial arteriolar and arterial calibre

5-HT concentration (M)	No. of vessels	% increase from base line calibre	Resting calibre (μm)	Mean arterial pressure (mmHg)
10^{-10}	7	8 ± 8	84 ± 13	117 ± 9
10^{-8}	14	40 ± 12	82 ± 9	116 ± 5
10^{-6}	21	36 ± 10	88 ± 11	101 ± 4
10^{-4}	12	38 ± 13	90 ± 13	107 ± 6

The data presented are the mean $\pm$ 1 S.E. of the mean. Only measurements made at mean arterial pressures greater than 80 mmHg are included. Over-all, fourteen cats were used.

In general the response, whether dilatatory or constrictory, was maximal approximately 15 sec after the administration of 5-hydroxytryptamine and the vessel calibre usually returned to base line levels within 120 sec following administration. In any single vessel, biphasic responses were not noted. There was no significant difference between the over-all dilatatory response at 5-hydroxytryptamine concentrations of 10^{-8}, 10^{-6} and 10^{-4} M.

The % change in calibre, following the perivascular application of 5-hydroxytryptamine, was plotted against the absolute resting calibre of the vessels for two concentrations of 5-hydroxytryptamine, viz. 10^{-8} and 10^{-6} M. The graphs are shown in Figs. 1 and 2.

The relationship between 5-hydroxytryptamine-induced changes in pial vessel calibre and the resting calibre of the vessel is best described by an exponential equation of the following form: $y = A - B.\log_e x$, where y = % change from resting vessel calibre and x = resting calibre. For both concentrations of 5-hydroxytryptamine there is a highly significant ($P < 0{\cdot}001$) relationship between the response to the amine and the resting vessel calibre and, by an analysis of variance between regressions, there is

no significant difference between this relationship for the two concentrations of 5-hydroxytryptamine that were examined. In other words, the small pial arterioles (< 70 μm in resting calibre) consistently and uniformly dilated in response to the perivascular application of 5-hydroxytryptamine; whilst with the larger pial vessels there was a tendency for there to be no change, or a constriction, following the administration of the

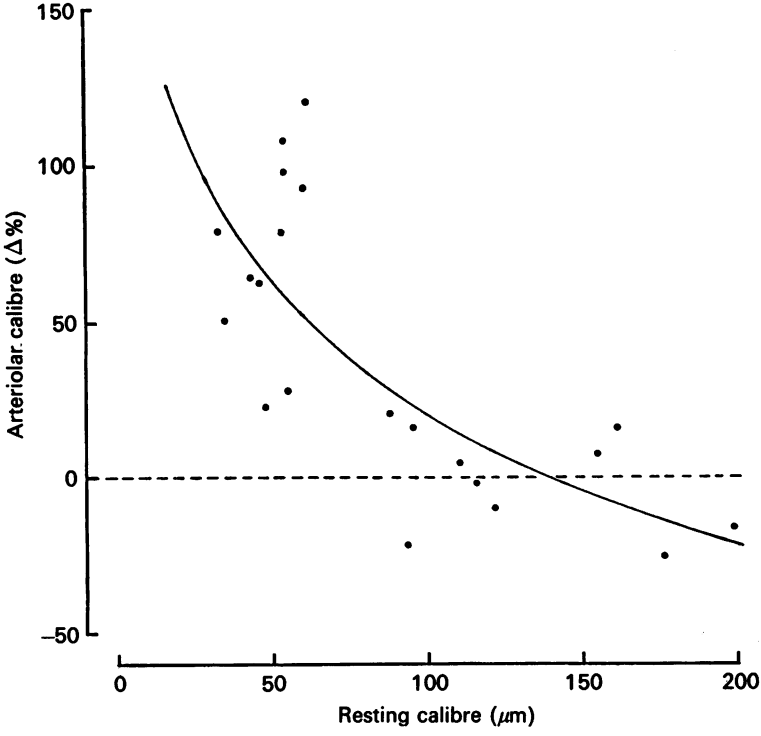

Fig. 1. The dependency of the pial arteriolar response to 10^{-6} M 5-hydroxytryptamine on the resting diameter of the vessels. The data best fit the following exponential regression: $[y+100]/100 = 1{\cdot}192-0{\cdot}594.\log_e[x]/100$, where y = the % change from base line calibre and x = base line calibre in μm. The correlation coefficient of the regression ($r = -0{\cdot}7360$) has a probability value of < 0·001. Only measurements made at mean arterial pressures > 80 mmHg have been included.

amine. The larger the resting calibre of a pial vessel, then the greater this tendency. The 5-hydroxytryptamine-induced responses were very similar at the two concentrations of 5-hydroxytryptamine studied.

In the cats, described hitherto, it was noted that there was a direct relationship between the magnitude of the 5-hydroxytryptamine-induced dilatation and the mean arterial pressure. This relationship was examined

further by subjecting five cats to graded hypotension, induced by haemorrhage, and the response of the pial vasculature to the microapplication of 5-hydroxytryptamine examined. The findings are presented in Figs. 3 and 4. In the smaller vessels (< 70 μm), there was a direct relationship between

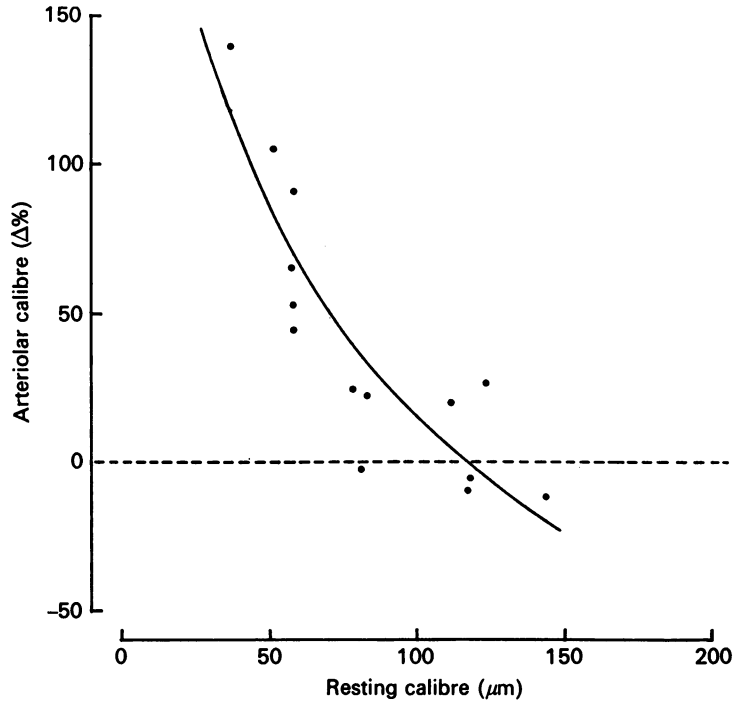

Fig. 2. The dependency of the pial arteriolar response to 10^{-8} M-5-hydroxytryptamine on the resting diameter of the vessels. The data best fit the following exponential regression: $[y+100]/100 = 1{\cdot}137 - 0{\cdot}988.\log_e[x]/100$, where y = the % change from base line calibre and x = base line calibre in μm. The correlation coefficient of the regression ($r = -0{\cdot}8823$) has a probability value of $< 0{\cdot}001$. Only measurements made at mean arterial pressures > 80 mmHg have been included.

the magnitude of the amine-induced increase in arteriolar calibre to the mean arterial blood pressure. Although there was no significant linear relationship between calibre changes and arterial pressure in the larger vessels ($\geqslant 70$ μm), there was a tendency for these vessels to show a more pronounced constriction at low arterial pressures (Fig. 4).

At no time did the microapplication of 5-hydroxytryptamine effect any significant changes in either mean arterial pressure or the arterial carbon dioxide tension.

DISCUSSION

The purpose of this investigation was to examine the direct cerebrovascular effects of 5-hydroxytryptamine *in vivo*. The technique of microinjection into the subarachnoid space was chosen because this method avoids the blood–brain barrier mechanisms to monoamines which lie at the

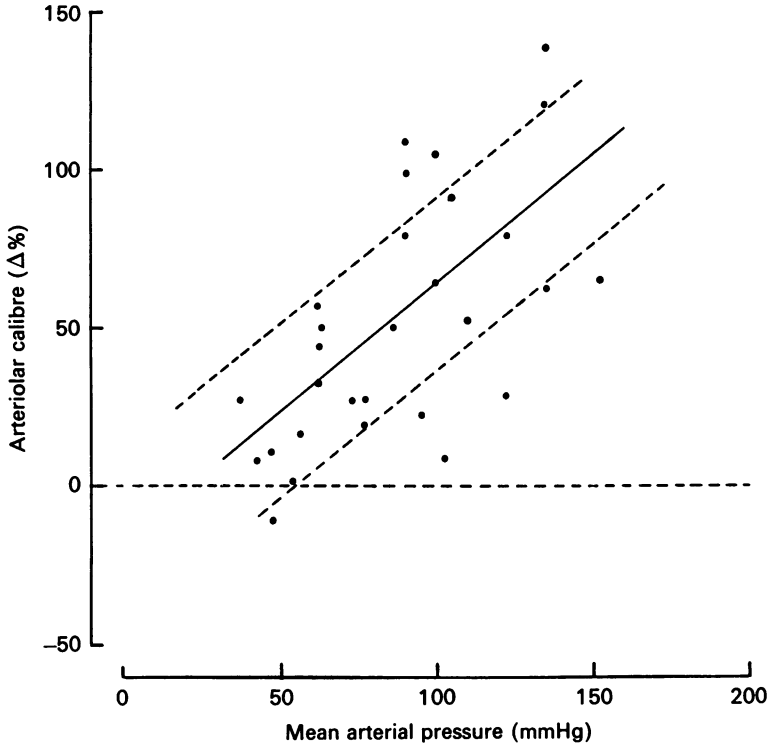

Fig. 3. The dependency of the response of pial vessels (< 70 μm) to 5-hydroxytryptamine (10^{-9} and 10^{-6} M) on mean arterial pressure. The data best fit the following linear regression: $y = A + Bx$, where y = % change from resting vessel calibre, and x = mean arterial pressure (mmHg). The correlation coefficient of the regression ($r = +0{\cdot}6347$) has a probability value of < 0·001. The $S_{y.x}$ ($\pm$ 29·41) is illustrated. The results are from nine cats in all, in five of which the blood pressure was lowered by controlled haemorrhage.

luminal surface of cerebral vessels (Bertler, Falck, Owman & Rosengrenn, 1966; Reese & Karnovsky, 1967). One major assumption was made, namely, that this approach affected primarily cerebrovascular smooth muscle, and that it was unlikely that the metabolism of the more distant, parenchymal cells was affected. To a certain extent, this assumption is substantiated by

the fact that 5-hydroxytryptamine, administered so as to bypass the blood–brain barrier, effects a reduction in cerebral metabolism (Harper & MacKenzie, 1977). The consequent decrease in cerebral blood flow is in complete contradistinction to the pial arteriolar dilatation, observed in the present study. The criticism could be levelled that the cerebrovascular actions of 5-hydroxytryptamine were mediated through a neural reflex;

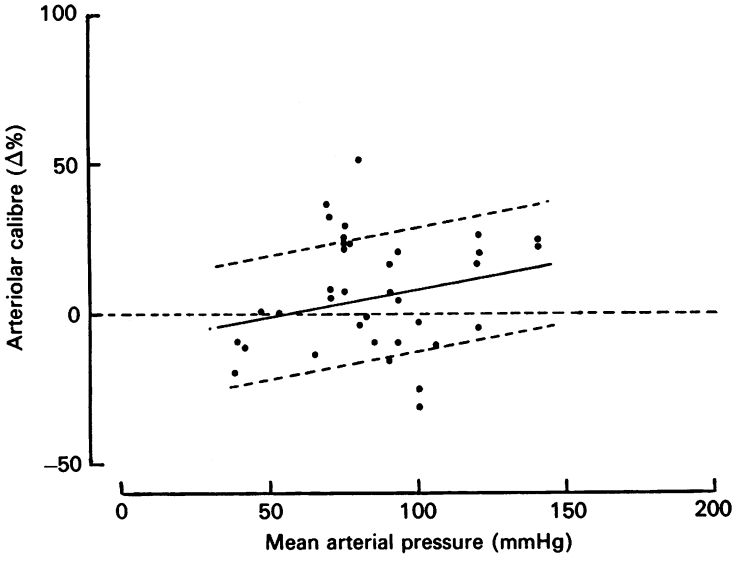

Fig. 4. The correlation between mean arterial pressure and the serotonin-induced dilatation in the larger pial arterioles and arteries (> 70 μm, resting calibre). The linear regression equation: $y = -10{\cdot}2 + 0{\cdot}178x$ is shown, where y = % change from resting vessel diameter and x = mean arterial pressure (mmHg). The correlation coefficient of the regression ($r = +0{\cdot}2990$) is not significant ($S_{y.x} = \pm 20{\cdot}49$). The results are from eleven cats in all, in five of which the blood pressure was reduced by controlled haemorrhage.

the cerebral vasculature is innervated by adrenergic, cholinergic and peptide-containing neurones as well as putatively sensory fibres (for review see Edvinsson & MacKenzie, 1976; Purves, 1972). However, a neural reflex is most improbable as *in vitro* studies (on functionally denervated vessels) have yielded results similar to those of the present investigation, viz. a 5-hydroxytryptamine-induced dilatation when isolated pial vessels are given active tone, and a 5-hydroxytryptamine-induced constriction in relaxed vessels (Edvinsson & Hardebo, 1976; Edvinsson, Hardebo & Owman, 1977). In the latter instance, the constriction has been shown to involve specific vascular receptors to 5-hydroxytryptamine.

The pial vascular dilatation, induced by 5-hydroxytryptamine, was

found to be inversely dependent on resting vessel calibre and directly dependent on mean arterial pressure. The common denominator could be vascular tone; and it is probable that the specific cerebrovascular actions of 5-hydroxytryptamine are largely tone-dependent. Based on observations in the dog forelimb, Haddy, Gordon & Emanuel (1959) have reported that 5-hydroxytryptamine induces a dilatation of arterioles and a constriction of large arteries, but that the over-all blood flow response was dependent on vascular tone, or 'total forelimb resistance'. Our current findings in the cerebrovascular bed are in complete agreement with these observations.

Relative constancy of cerebral blood flow is maintained in the face of moderate changes in perfusion pressure, a phenomenon that is sometimes termed autoregulation (Harper, 1966; Fitch, MacKenzie & Harper, 1975). This constancy is achieved by a dilatation of the cerebral resistance vessels as perfusion pressure is decreased (or, in this instance, as mean arterial pressure is reduced), and a constriction of the cerebral resistance vessels as perfusion pressure is increased. Thus, at low arterial pressures there is a reduction in cerebrovascular resistance, or reduction in vascular tone, and this is associated with the decreased responsiveness of the pial arterioles to 5-hydroxytryptamine, observed in the present study. For pial vessels of resting diameter between 24 and 200 μm in resting diameter, the average dilatation is 0·8%/mmHg in response to an arterial pressure reduction, induced by graded haemorrhage, from 120 to 35 mmHg (unpublished observations).

The most pronounced dilatatory responses were seen in those arterioles $< 70\ \mu$m in resting calibre. These vessels have a markedly greater wall/lumen ratio than the larger arterioles (Mchedlishvili, Baramidze, Nikolaishvili & Mamisashvili, 1974). The % change of the small arterioles in response to either induced hypotension or hypertension is greatest in the arterioles $\sim 50\ \mu$m in resting calibre (Mchedlishvili *et al.* 1974; MacKenzie *et al.* 1976). The greatest fraction of total cerebrovascular resistance is sited at the smaller pial and parenchymal arterioles (Stromberg & Fox, 1972; Symon, Held & Dorsch, 1973), this being no different from the pattern found in most peripheral vascular beds (for review: Folkow & Neil, 1971). Thus, the 5-hydroxytryptamine-induced dilatation was maximal in those vessels that have the greatest vascular tone in the pial circulation.

This concept, that the cerebrovascular actions of 5-hydroxytryptamine are tone-dependent, can explain a number of observations in the literature. An *in vitro* study on the responsiveness of the larger feline intracranial arteries demonstrated that 5-hydroxytryptamine could contract these vessels in a dose-related manner (Nielsen & Owman, 1971). However, later investigations have noted that 5-hydroxytryptamine can elicit a dose-

related dilatation, provided that the isolated cerebral vessels are given active tone by the addition of an agent such as prostaglandin $F_{2\alpha}$, (Edvinsson & Hardebo, 1976; Edvinsson *et al.* 1977).

The 5-hydroxytryptamine-induced reduction in regional cerebral blood flow is enhanced during hypercapnia (Deshmukh & Harper, 1973) and diminishes greatly during hypocapnia (Ekström-Jodal, von Essen, Häggendal & Roos, 1974), when cerebral resistance vessels are respectively dilated and constricted.

The tone-dependent actions of 5-hydroxytryptamine explain why the amine's constrictory effect is enhanced in instances of experimental cerebral ischaemia and infarction (Bell, Sundt & Nofzinger, 1967; Raynor, McMurty & Pool, 1961). In such pathological instances the tone of the cortical vessels will be low, due to a combination of local hypoxia and acidosis as well as a focal reduction in perfusion pressure.

Our findings of a direct 5-hydroxytryptamine-induced dilatation are contrary to previous studies which have demonstrated a vasoconstrictory action of 5-hydroxytryptamine on the pial vasculature (Bell *et al.* 1967; Raynor *et al.* 1961). However, these studies involved the flushing of 5-hydroxytryptamine solutions over the entire cerebral cortex with the pial vascular changes being examined some minutes later, as opposed to the limited perivascular microinjection technique of the present study. Accordingly, it is probable that the 5-hydroxytryptamine-induced reduction in arteriolar calibre, observed in earlier studies, was secondary to a suppression of cortical metabolism or electrocortical activity (Harper & MacKenzie, 1977).

When our previous study (Harper & MacKenzie, 1977) is viewed alongside the present investigation, it becomes clear that the cerebral circulatory actions of 5-hydroxytryptamine are diverse and complex. A large number of factors will determine the overall cerebral circulatory response to 5-hydroxytryptamine, and the relevance of each of these factors should be assessed in each differing experimental situation. The status of the blood–brain barrier, or of the monoaminergic mechanisms within the blood–brain barrier, is of primary importance when examining the effects of systemic 5-hydroxytryptamine. This amine can depress either brain metabolism or electrocortical function, but the balance between parenchymal and vascular actions of 5-hydroxytryptamine could vary with, for example, depth of anaesthesia. The direct vascular effects are dependent on vessel tone, an all-embracing concept. Not only does vascular tone vary according to the segment of the cerebrovascular bed under consideration, but also the tone of each individual segment can be affected to differing degrees by a number of physiological variables such as arterial pressure, blood gas tensions and the activity of sympathetic vasomotor fibres.

These relationships clearly deserve further investigation before there is an understanding of any physiological role for 5-hydroxytryptamine in the cerebral circulation.

We are grateful to the Wellcome Trust and to Tenovus (Scotland) who supported these experiments, and to the staff of the Wellcome Surgical Research Institute for their expert technical assistance, in particular Mrs Margaret Stewart. We would also like to extend our thanks to Dr M. Wahl and Dr W. Kuschinsky who allowed one of us (E.T. MacKenzie) to visit their laboratory and demonstrated their techniques for investigating the pial vasculature.

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