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Role of 5HT in the morbidity of cerebral infarction—a study in the gerbil stroke model

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SUMMARY Cerebral infarction was produced by unilateral carotid ligation in the gerbil, and 5HT levels in the cerebral hemispheres were assayed 3·5 hours later. A bilateral fall was confirmed, with the greatest change occurring on the side of carotid ligation in animals showing the clinical sequelae of infarction. Neither absolute levels nor right left differences in 5HT content related directly to the nature or prevalence of neurological morbidity. Neither putative 5HT receptor antagonists nor agents causing increasing brain 5HT levels produced consistent changes in the prevalence of neurological morbidity. It is argued that the fall in 5HT in a cerebral infarct is more likely to be due to reduced synthesis and turnover than to release of the amine into the synaptic cleft. These findings cast doubt on the hypothesis that a significant part of the morbidity and mortality of cerebral infarction is due to the sequelae of 5HT release.

It has been suggested that changes in neurotransmitters in ischaemic brain have secondary deleterious effects on cerebral blood flow and metabolism.[1] In particular the levels of 5-hydroxytryptamine (5HT) have been shown to fall in experimental models of cerebral ischaemia,[2 3] and are low also in human cerebral infarcts at autopsy.[4] In order to investigate the possible relevance of 5HT changes to the clinical sequelae of cerebral infarction, and to explore the effects of pharmacological intervention, a further study in the gerbil stroke model has been carried out.

Methods

Cerebral ischaemia was produced by unilateral ligation of the common carotid artery through a midline cervical incision under pentobarbitone (50 mgm/Kg ip) anaesthesia. Sham operated animals served as controls.

Clinical signs of cerebral infarction were elicited on recovery from anaesthesia. Animals were classed as showing normal behaviour, as having splayed contralateral limbs, or as having been seen to be circling or to have rolling seizures.

Some animals were killed by decapitation at 3·5 hours after surgery for assay of cerebral hemisphere 5HT content. The brain was rapidly removed and placed on a cooled plate. The right and left cerebral hemispheres were then dissected, weighed and separately homogenised prior to assay by the method of Curzon and Green.[5] Other animals were followed for 24 hours to confirm the clinical observations made in the first post-operative hours and then killed for histological examination (Dr P Lantos).

To study the relevance of 5HT levels, groups of animals were given (by intraperitoneal injection) the following materials designed to depress or elevate whole brain 5HT, or to block 5HT receptors.

5-hydroxy-l-tryptophan[6]	150 mgm/Kg 1 hour before operation
L-tryptophan	150 mgm/Kg 1 hour before operation
after Pargyline[7]	7·5 mgm/Kg 3 hours previously
Quipazine[8]	15 mgm/Kg 1 hour before operation
Methergoline[7]	5 mgm/Kg 1 hour before operation
Methysergide[7]	10 mgm/Kg 1 hour before operation
BW 501C[9]	10 mgm/Kg 1 hour before operation
Cyproheptidine[10]	1 mgm/Kg 1 hour before operation 10 mgm/Kg 1 hour before operation
p-chlorophenylalanine(pCPA)[11]	300 mgm/Kg 48 hours and 24 hours after operation

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Some treated animals were killed at 3·5 hours after surgery for cerebral 5HT assay, and others at 24 hours for histological examination. Both groups were assessed clinically for evidence of neurological deficit prior to sacrifice.

Results

The left and right hemisphere 5HT levels 3·5 hours after right carotid ligation and in sham operated animals are shown in table 1. Although there was a bilateral fall, detectable even in clinically unaffected animals, the maximal change was in affected animals ipsilateral to the ligation. A significant right-left difference in 5HT content was seen only in the neurologically affected group.

The results in table 2 suggest that the fall of 5HT in the right hemisphere occurred irrespective of the behavioural abnormality noted. The change did not appear to be limited to those animals exhibiting rolling seizures. The numbers were small, however, and none of the differences for individual subgroups attained statistical significance.

The 5HT levels after the various drug treatments are shown in the figure. As expected, levels in sham operated animals were elevated after preloading with L-tryptophan after pargyline, and after 5-hydroxy-l-tryptophan, and were depleted after 48 hours administration of pCPA which inhibits tryptophan hydroxylase. (The rather modest elevation of 5HT after L-tryptophan may in retrospect be attributable to the small dose of monoamine oxidase inhibitor employed). The putative 5HT antagonists methysergide, BW 501C and cyproheptidine had no effect on 5HT level. Quipazine and methergoline both increased hemisphere 5HT content.

The effect of surgery was obvious in nearly all the treated groups with an approximately 30% fall in 5HT content in the ipsilateral hemisphere of clinically affected animals. The exceptions were in the group given 5-hydroxy-l-tryptophan in whom the fall (from a very high level) was small and for those given pCPA where the fall (from a lower than normal level) was greater (approximately 50%).

The relationship of the change in 5HT brought about by the drugs, and the number of animals showing neurological deficit over 3·5 hours is shown in table 3. The only significant departure

Table 1 *Level of 5HT in cerebral hemispheres 3·5 hours after ligation of the right carotid artery related to neurological findings*

Group	*n*	*Level of 5HT ng/g±SD*		
		R hemisphere	*R v L (Student t)*	*L hemisphere*
Sham operation	13	913± 94	NS	887±119
Operated unaffected	19	789±116†	NS	795±124*
Operated affected	21	622±107†	p<0·005	759±119†

*p<0·05 } with reference to level on same side in sham operated
†p<0·005 } animals (student *t*).

Table 2 *Fall in cerebral 5HT after ligation of the right carotid artery related to nature of neurological deficit observed*

Group		*Level of 5HT ng/g±SD*	
		R hemisphere	*L hemisphere*
Splayed limbs	(4)	618±130	758±156
Circling	(9)	639±102	739±132
Seizures	(8)	604±113	782± 94

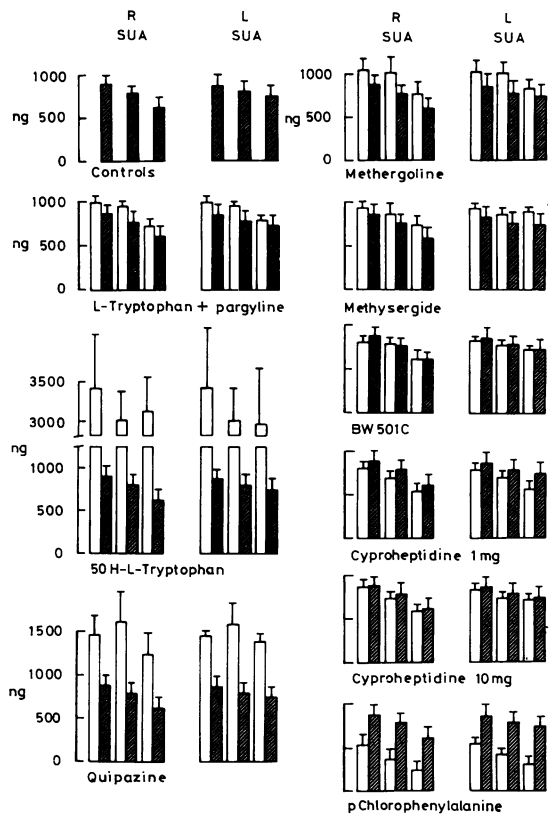

Figure *5HT content of cerebral hemispheres 3·5 hours after R carotid ligation or sham operation and the effect of pre operative drugs. 5HT in ng/g±SD. R=right; L=left; S=sham operation; U=operated, unaffected; A=operated, affected. Values for controls (hatched columns) reproduced in each case for comparison. Scale where unmarked as for controls.*

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from the control data was the reduced neurological morbidity in BW 501C treated animals. The proportion of animals affected by neurological deficit at 3·5 hours was 50% for controls (n=40), 40% in those given agents causing elevated levels of 5HT (n=72) and 26% in those given methysergide, BW 501C or cyproheptidine (n=87). This last reduction was significant at $p<0.05$ ($\chi^2=6.8$).

Right–left differences in hemispheric levels of 5HT showed no simple correlation with prevalence of neurological signs in the different groups. High or low proportions of affected animals were seen with or without a significant right left difference (table 4).

After the more extended period of neurological observation (24 hours) more animals were affected with a higher mortality after L-tryptophan and quipazine. None of the other agents caused a significant departure from the control data (table 5). The combined mortality for all those animals given agents causing elevated brain 5HT (n=113) was 44% (controls 18% $p<0.01$). The proportion with neurological signs, fatal or not, was 62·5% (controls 31% $p<0.001$). The combined morbidity and mortality among groups given methysergide, BW 501C or cyproheptidine (n=104) was unchanged from control values (mortality 16%, combined morbidity and mortality 34%). Histological examination showed evidence of infarction in 32 of 33 clinically affected animals, and in none of 10 animals whose motor behaviour was normal.

Combining the 3·5 hour and 24 hour data reveals an overall morbidity and mortality in controls of 40·5% (n=79). With elevated 5HT (L-tryptophan, 50H-L-tryptophan, quipazine, methergoline; n=185) 53% were affected ($\chi^2=3.4$ ns).

Table 3 *Effect of drugs on 5HT content of R hemisphere and on neurological morbidity 3·5 hours after ligation of R carotid artery*

Drug	*n*	*5HT level % change from control level*			*Neurological morbidity (%)*
		Sham op	*Op unaffected*	*Op affected*	
Controls	53	—	—	—	50
L-tryptophan + pargyline	15	+12*	+25*	+23*	41
5-OH-L-tryptophan	29	+294*	+292*	+432*	35
Quipazine	13	+ 64*	+110*	+101*	42
Methergoline	21	+ 15*	+ 31*	+ 24*	42
Methysergide	35	+ 5	+ 10*	+ 14*	26
BW501C	35	− 8	+ 10	0	18†
Cyproheptidine 1 mgm/kg	20	− 10	− 11	− 8	36
Cyproheptidine 10 mgm/kg	23	− 5	− 5	− 1	33
pCPA	35	− 36*	− 52*	− 58	38

*p < 0·05 Student *t* cf controls †p < 0·01 chi square

Table 4 *Effect of drugs on R/L differences in hemisphere 5HT content and on neurological morbidity 3·5 hours after ligation of R carotid artery*

Drug	*n*	*R/L differences*			*Neurological morbidity (%)*
		Sham op	*Op unaffected*	*Op affected*	
Control	53	—	—	++	50
L-tryptophan and pargyline	15	—	—	—	41
50H-L-tryptophan	29	—	—	—	35
Quipazine	13	—	—	—	42
Methergoline	21	+	—	—	42
Methysergide	35	—	—	+	26
BW501C	35	—	—	—	18*
Cyproheptidine 1 mgm/kg	20	—	+	+	36
Cyproheptidine 10 mgm/kg	23	—	—	+	33
pCPA	35		++	++	38

+p < 0·05 ++p < 0·005 Student *t* *p < 0·01 chi square of controls

Table 5 *Mortality and combined morbidity and mortality 24 hours after unilateral carotid ligation*

Treatment group	*n*	*Mortality (%)*	*Total morbidity and mortality (%)*
Control	39	18	31
L-tryptophan	29	55*	76*
5-OH-L-tryptophan	24	37·5	62·5
Quipazine	34	47*	67·5*
Methergoline	26	44	42
Methysergide	19	16	53
BW501C	25	20	32
Cyproheptidine 1 mgm/kg	32	12·5	25
Cyproheptidine 10 mgm/kg	28	16	36
pCPA	17	41	59

*p < 0·01 cf controls

With receptor antagonists or depletion of brain 5HT. (Methysergide, BW 501C, cyproheptidine and pCPA; n=237) the combined morbidity and mortality was 34% ($\chi^2=1.2$ ns).

Discussion

The data confirm earlier reports of a striking and significant fall in cerebral 5HT content after unilateral carotid ligation in the gerbil.[2 3] Although the changes are bilateral the greatest fall is seen in the hemisphere ipsilateral to the carotid ligation, and in animals that show abnormal motor behaviour. A fall is seen whether the animals show splaying of limbs, circling or seizures. The presence of these abnormalities correlates very closely with histological evidence of infarction. It can be concluded that the 5HT change is related to infarction rather than to seizure activity (which has been shown to be the major cause of changes in dopamine and norepinephrine levels in this model).

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It has been suggested that the fall in 5HT accompanying infarction is due to release into the synaptic cleft where it may have behavioural sequelae, and/or into the vicinity of blood vessels where it might aggravate the extent and depth of ischaemia by provoking secondary vasoconstriction. In a previous study we showed that cerebral 5HIAA levels had fallen 3·5 hours after carotid ligation in the gerbil,[2] and we argued that reduced activity in median raphe serotinergic neurones could explain the bilateral change in 5HT and its metabolite. There was no need to invoke synaptic release. The present finding that a 30% fall in 5HT occurs even after L-tryptophan and pargyline favours the view that 5HT synthesis is impaired. The finding that the post operative fall is largely overridden by pre-loading animals with 5-hydroxy-l-tryptophan can be taken as further evidence that the fall in 5HT due to infarction is specific to changes in serotonergic neurones. Low 5HT and 5HIAA levels also have been found in acute cerebral infarcts in man, together with elevated levels of tryptophan in the necrotic area.[4] These changes again support the concept of reduced synthesis and turnover of 5HT in infarcted tissue. In perifocal oedematous white matter tryptophan, 5HT and 5HIAA levels were all elevated, perhaps reflecting impaired transport out of the infarcted area. If impaired synthesis, rather than release, is the cause of the fall in 5HT the clinical relevance of the change might not be as important as claimed previously.

It seems unlikely from the results of the present study that either absolute levels of 5HT or right–left differences in hemisphere content have a major influence on the motor behaviour of the animals after carotid ligation. Similarly receptor antagonists, including those with central effects failed to produce a striking change in the prevalence of neurological morbidity. Although a small but significant reduction in neurological changes was seen with BW 501C and with the group of putative receptor antagonists as a whole at 3·5 hours, the combined data including the 24 hour clinical observations showed no such difference. Again though some increased mortality accompanied 5-hydroxytryptophan and L-tryptophan in the 24 hours observation period, the combined data with larger numbers showed no significant exacerbation of clinical sequelae by these drugs.

The conclusion of the present series of experiments must be that there is no simple link between 5HT levels in ischaemic brain and clinical sequelae. Only a clinical trial, however, could show if a 5HT receptor antagonist could influence the outcome of acute cerebral infarction in man.

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