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Measurement of 5-Hydroxytryptamine Turnover Rate in Rat Cerebral Arteries

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Abstract—Two methods for assessing 5-hydroxytryptamine (5-HT) turnover rate have been tested in the cerebral vessels of the rat. The pretreatment of the animals with benserazide 45 min before death brought about an increase in the levels of 5-hydroxytryptophan (5-HTP). When the MAO inhibitor pargyline was injected and the animals killed at different times, there was an exponential decrease in the concentration of 5-hydroxyindole acetic acid (5-HIAA) with time, whereas the increase in 5-hydroxytryptamine was linear only during the first 30 min, thereafter reaching a plateau. This pattern was similar to that obtained in the caudate nucleus after MAO blockade. In the hippocampus, pargyline induced a lineal accumulation of 5-HT throughout the experiment as well as an exponential decay of the 5-HIAA concentration. These results indicate that the turnover rate of 5-HT can be appraised in the rat cerebral arteries either after the rate of disappearance of 5-HIAA or from the accumulation rate of 5-HTP.

5-Hydroxytryptaminergic nerve endings have been found in the cerebral blood vessels of several animal species, including man (Reinhard et al 1979; Griffith et al 1982; Sano et al 1982; Edvinsson et al 1983; Griffith & Burnstock 1983; Marco et al 1985; Scatton et al 1985; Alafaci et al 1986). The cell bodies of this innervation are located in the raphe nuclei of the brainstem (Reinhard et al 1979; Edvinsson et al 1983) though in some instances they can also be found in the sympathetic superior cervical ganglia (Marco et al 1985; Alafaci et al 1986). The function of this innervation remains unclear but it might contribute to the maintenance of the cerebral blood flow taking into account that 5-hydroxytryptamine (5-HT) can induce a potent vasoconstrictor response in this vasular bed (Urquilla et al 1975).

The turnover rate of a neurotransmitter is believed to be an indicator of the activity of the neuron containing it (Costa & Neff 1970). Methods to appraise 5-HT turnover rate in the central nervous system have been developed (Neff et al 1969). Some are based on the blockade of a step in the biosynthetic pathway of 5-HT and then measurement of its turnover rate either from the rate of accumulation of the substrate or from the rate of disappearance of the reaction product. Thus, it is possible to measure i) the rate of accumulation of 5-hydroxytryptrophan (5-HTP) after the inhibition of L-aromatic amino acid decarboxylase, ii) the rate of accumulation of 5-HT or disappearance of 5-hydroxyindoleacetic acid (5-HIAA) when monoamine oxidase is inhibited, or iii) the rate of accumulation of 5-HIAA after blockade of the transport of acidic compounds with probenecid.

We have used the routes i and ii above with cerebral arteries of the rat to ascertain that best suited to measure the turnover rate of 5-HT in those blood vessels.

Materials and Methods

Male Sprague-Dawley rats, 100–130 g, were given benserazide (800 mg kg^{-1} i.p.) 45 min before being decapitated. Other rats were given pargyline (75 mg kg^{-1} i.p.) and were divided into four groups that were killed after 15, 30, 45, and 60 min, respectively. In all cases, a group of untreated rats was also used, and considered as the 0 min group.

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After the animals were killed the brain was quickly removed and the circle of Willis with its branches dissected out. For those rats treated with pargyline the caudate nucleus and the hippocampus from the right side were also removed. The tissues were frozen on dry ice and kept at −15°C.

The tissues samples of each animal were homogenized separately in 0·4 M $HClO_4$ with 0·002% (w/v) ascorbic acid, sonicated and centrifuged at 12000 rev min^{-1} for 2 min. 5-HT and metabolites were assayed in 50 μL aliquots of supernatants by high pressure liquid chromatography (HPLC). Protein in the precipitates was measured according to Lowry et al (1951) and related to the final concentrations of hydroxyindoles. The HPLC system consisted of a pump (Meek 1976) giving a flow rate of 0·8 mL min^{-1}, a Supelco sampling valve, and a reverse phase column (BIO-SIL ODS-10, BIO-RAD) with a guard column (Bondapak C_{18}/Corasil, Waters). The hydroxyindoles were detected with an electrochemical detector (LC-4A, Bioanalytical Systems) and a glassy carbon electrode set at +0·5 V vs an Ag/AgCl reference electrode. The mobile phase was prepared according to Lacković et al (1981) with slight modifications when 5-HTP was assayed to get a good separation from the front peak.

The turnover rate of 5-HT was calculated from the rate of disappearance of 5-HIAA according to Neff et al (1969) as follows:

$$TR_{5\text{-HT}} = k \times [5\text{-HIAA}]_0$$

where k = decay rate constant and $[5\text{-HIAA}]_0$ = initial concentration of 5-HIAA.

Statistical analysis of the results was made using Student's *t*-test (Snedecor & Cochran 1980) when two samples were compared, or by the Peritz' F test (Harper 1984) if more samples were compared. The data were fitted according to Barlow (1983).

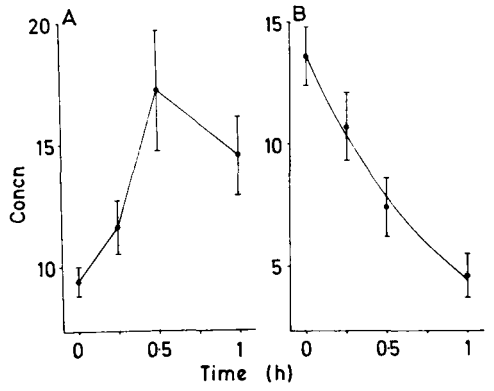

FIG. 1. Time course variations in the concentration (pmol mg^{-1} protein) of 5-HT (A) and 5-HIAA (B) in rat cerebral arteries after pargyline. The points represent the average of 2 experiments with 5–6 animals each. Bars are the means with s.e.m.

Pargyline hydrochloride, 5-hydroxytryptamine creatinine sulphate, and 5-hydroxy-L-tryptophan were purchased from Sigma. Benserazide was a gift from Roche.

Results

Effect of benserazide on the levels of 5-HTP in rat cerebral arteries

Benserazide administered 45 min before death significantly increased the 5-HTP concentration in the cerebral blood vessels ($7{\cdot}5 \pm 0{\cdot}6$ pmol (mg protein)$^{-1}$ $n = 12$) compared with control animals ($4{\cdot}4 \pm 0{\cdot}4$ pmol (mg protein)$^{-1}$ $n = 12$) $P < 0{\cdot}001$.

Effect of pargyline on the levels of 5-HT and 5-HIAA in the cerebral arteries, caudate nucleus, and hippocampus of the rat

The intraperitoneal administration of pargyline induced a significant increase in the 5-HT content of the rat cerebral blood vessels which was linear during the first 30 min ($r = 0{\cdot}971$) and reached a plateau after 1 h (Fig. 1). The levels of 5-HIAA in the same tissues showed an exponential decay ($r = 0{\cdot}995$, Fig. 2), with a fractional constant rate of $1{\cdot}123 \pm 0{\cdot}079$ h^{-1} (Table 1). Under these experimental conditions the concentration of 5-HIAA at 0 time was $13{\cdot}7 \pm 0{\cdot}4$ pmol (mg protein)$^{-1}$ (Table 1). The value of the turnover rate determined from the rate of disappearance of 5-HIAA was 15·3 pmol (mg protein)$^{-1}$ h^{-1} (Table 1).

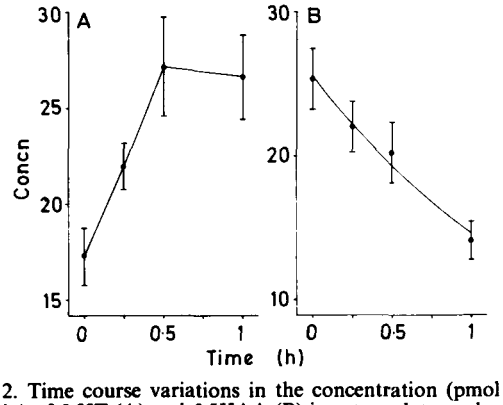

FIG. 2. Time course variations in the concentration (pmol mg^{-1} protein) of 5-HT (A) and 5-HIAA (B) in rat caudate nucleus after pargyline. The points represent the average of 2 experiments with 5–6 animals each. Bars are the means with s.e.m.

Table 1. 5-HIAA steady-state concentration, constant rate, and 5-HT turnover rate in cerebral vessels, caudate nucleus and hippocampus of the rat.

Tissue	$(5\text{-HIAA})_0 \pm$ s.e.m. (pmol (mg protein)$^{-1}$)	$K \pm$ s.e.m. (h^{-1})	$TR_{5\text{-HT}}$ (pmol mg^{-1} h^{-1})
Cerebral arteries	$13{\cdot}7 \pm 0{\cdot}4$	$1{\cdot}123 \pm 0{\cdot}079$	15·3
Caudate nucleus	$25{\cdot}5 \pm 0{\cdot}6$	$0{\cdot}557 \pm 0{\cdot}056$	14·2
Hippocampus	$17{\cdot}7 \pm 1{\cdot}0$	$0{\cdot}783 \pm 0{\cdot}148$	13·7

These results are the average of 2 experiments with 5–6 animals each.

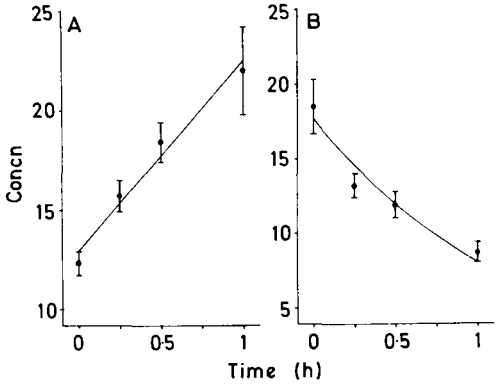

FIG. 3. Time course variations in the concentration (pmol mg^{-1} protein) of 5-HT (A) and 5-HIAA (B) in rat hippocampus after pargyline. The points represent the average of 2 experiments with 5–6 animals each. Bars are the means with s.e.m.

A similar behaviour in the variation of the content of 5-HT and 5-HIAA with time was see in the caudate nucleus after the injection of pargyline. The accumulation of 5-HT was linear for the first 30 min ($r = 0{\cdot}9997$) after which it levelled out (Fig. 2). The concentration of 5-HIAA decreased exponentially with time ($r = 0{\cdot}987$) at a constant rate of $0{\cdot}557 \pm 0{\cdot}056$ h^{-1}, the initial concentration being $25{\cdot}5 \pm 1{\cdot}3$ pmol (mg protein)$^{-1}$ (Table 1).

The turnover rate of 5-HT calculated from these data was 14·2 pmol (mg protein)$^{-1}h^{-1}$ (Table 1).

In the hippocampus, pargyline caused a linear increase in the concentration of 5-HT ($r = 0{\cdot}988$) throughout the experiment whereas the content of 5-HIAA diminished exponentially ($r = 0{\cdot}956$) (Fig. 3). With the 5-HIAA the fitted data gave an initial concentration of $17{\cdot}7 \pm 1{\cdot}0$ pmol (mg protein)$^{-1}h^{-1}$ and a rate constant of $0{\cdot}783 \pm 0{\cdot}148$ h^{-1} (Table 1). The value of the resulting turnover rate of 5-HT was 14·7 pmol (mg protein)$^{-1}h^{-1}$ (Table 1).

Discussion

In the present report two methods of assessing 5-HT turnover rate have been tested on the cerebral blood vessels of the rat to establish the function of the 5-HT-ergic innervation of that vascular bed. The results indicate that both methods can be used.

Benserazidine, after 45 min, induced an increase in the concentration of 5-HTP, while pargyline brought about both an accumulation of 5-HT and a decrease in the 5-HIAA content with time. However, the blockade of the L-aromatic amino acid decarboxylase was less effective in our hands as a means of measuring the 5-HT turnover in the cerebral arteries as it was technically more cumbersome and a high dose of a commercially unavailable drug was needed to inhibit the enzyme. This inhibits the use of that procedure for the study of the accumulation of 5-HTP with time although it would give a qualitative hint of the activity of the 5-HT innervation if the accumulation of 5-HTP were measured after a single period of time (Marco & Meek 1979).

The use of the MAO inhibition to assess 5-HT turnover rate is based mainly upon the assumptions that the synthesis of 5-HT equals its degradation and that the only pathway involved in the removal of the amine is its deamination to 5-HIAA (Neff et al 1969). Therefore, the turnover of 5-HT should be obtained from the rate of its accumulation or from the rate of disappearance of 5-HIAA. However, in the present experiments these hypotheses only partially held. Indeed, the increase in 5-HT concentration with time in rat cerebral arteries was linear only for the first 30 min, thereafter the curve levelled out, indicating that after doubling of the initial concentration there is either an inhibition of the synthesis of 5-HT or the activation of another mechanism other than deamination responsible for its removal (Millard et al 1972). This effect might be more widespread for it also happened in caudate nucleus. This could explain the failure of this method to show a decrease in 5-HT turnover rate (Marco & Meck 1979). This would be difficult to detect when the accumulation of 5-HT has been slowed down already. Since the accumulation of 5-HT with time seems to have several components, its use in measuring 5-HT turnover in rat cerebral arteries is not recommended, unless the period of time in which the accumulation of the amine reflects exactly its rate of synthesis is well established.

The decline of 5-HIAA after inhibition of monoamine oxidase seems to be a better choice for assessing 5-HT turnover in rat cerebral arteries. The variation with time was exponential, indicating that it was due to a single process. Moreover, the results were obtained when the initial 5-HT concentration was at normal values. Therefore, the amine content at the time of MAO inhibition depended only on its rate of synthesis, without any other interfering mechanism such as feedback inhibition or a different 5-HT removal pathway being involved. The same results were obtained in the caudate nucleus and hippocampus.

The turnover rate of 5-HT measured from the rate of disappearance of 5-HIAA after MAO blockade was similar in all three tissues studied. This suggests that the overall activity of the 5-HT innervation of rat cerebral arteries is no different from that shown by the 5-HT innervation impringing on the caudate nucleus or hippocampus. The only difference might be the individual activity of the neurons acting on the cerebral blood vessels. They seem to be more active as they need less time to renew their content of neurotransmitter.

Although the appraisal of 5-HT turnover rate by means of these techniques is well established in studies involving the CNS, this is the first attempt to apply them to the cerebral circulation. They might represent a useful tool in the elucidation of the functions of the 5-HT innervation present in cerebral arteries.

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