electron microscopy after a superfusion experiment with L-[3,5-3H]tyrosine, revealing that the radioactivity was distributed in a superficial layer of well-preserved tissue, 500 μm in depth. Despite the development of a highly sensitive radioenzymic method allowing the detection of as little as 25 pg of dopamine, the isotopic method (L-[3,5-3H]tyrosine) still presents some advantages; indeed, its sensitivity is higher and it allows the simultaneous study of synthesis and release processes. In any case the similarity of the results obtained in isotopic and non-isotopic studies will be of great help in further establishing the properties of the release process in this dopaminergic pathway.

In addition the dopaminergic system, the caudate nucleus is also innervated by cholinergic and serotoninergic neurons which are involved in extrapyramidal processes. The cup technique has already been used successfully to study the release of acetylcholine (Jones et al., 1973) and 5-hydroxytryptamine (Ternaux et al., 1975) in physiological and pharmacological studies. Most of our experiments involved the use of acute preparations; however, the cup technique has also been adapted to chronic studies. The spontaneous and amphetamine-induced release of newly synthetized 3H-labelled dopamine could be observed for periods as long as 5 days in the unanaesthetized monkey restrained in a chair (Gauchy et al., 1974).

The results of recent efforts to evaluate the release of transmitters in the central nervous system have been encouraging. It is hoped that this progress will stimulate further studies in this area.

Jones, B. E., Guyenet, P., Chéramy, A., Gauchy, C. & Glowinski, J. (1973) Brain Res. 64, 355-369

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Monoamine Metabolism in the Isolated Perfused Rat Brain

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An advantage of isolated-perfused-organ techniques is that they enable the investigator to perform metabolic studies on an organ in a situation where he has a high degree of control over the experimental conditions and in which the structural integrity of the tissue is preserved.

When compared with the body of published information concerning the metabolism of perfused liver or kidney there has been little work carried out with the perfused brain. This in part results from the technical difficulties encountered in isolating those branches of the carotid-arterial tree which supply only intracranial structures from those which supply other tissues of the head.

1976
A small amount of work has been published concerning monoamine metabolism in the brain. The uptake and metabolism of L-dopa (3,4-dihydroxyphenylalanine) was studied by Horst & Jester (1971) and Horst et al. (1973), and Krieglstein & Niemeyer (1975) reported the maintenance of usual concentrations of 5-hydroxytryptamine, dopamine (3,4-dihydroxyphenethylamine) and noradrenaline during a 30 min perfusion with a basal medium. The synthesis of 5-hydroxytryptamine at a rate linear with time during a 2h perfusion was reported by Graham et al. (1975).

One of the crucial tests of the acceptability of perfused-organ techniques is to ascertain whether the metabolic properties of the perfused organ are comparable with those of the organ in vivo. In the present paper we discuss this aspect of organ perfusion in relation to monoamine metabolism in the isolated perfused rat brain.

Methods

The brains of adult male Wistar rats (250–300 g) were perfused by using the method described by Woods et al. (1974) with a semi-synthetic medium consisting of dialysed bovine serum albumin (8 g/100 ml) washed aged human erythrocytes (final haematocrit 25%) and the bicarbonate-buffered saline of Krebs & Henseleit (1932).

After perfusion, brains were rapidly removed from the skull, frozen and stored at −20°C until analysis. The following analytical methods were used: 5-hydroxytryptamine and 5-hydroxyindol-3-yl acetic acid (Curzon & Green, 1970); L-tryptophan (Denckla & Dewey, 1967); dopamine and noradrenaline (Chang, 1964); L-tyrosine (Waalkes & Udenfriend, 1957); L-phenylalanine (McCaman & Robins, 1962).

Results and discussion

(a) General properties of the preparation. As has been previously reported (Woods et al., 1974), the structure of the brain, as judged by light and electron microscopy, is well preserved during 2h of perfusion, and the rates of glucose metabolism and acetoacetate metabolism are very similar to those reported in vivo as determined by arteriovenous concentration differences (see Table 1).

(b) Monoamine and amino acid contents of brain during perfusion with a basal medium. Brains were perfused for periods up to 2h with a basal medium, and the contents of

Table 1. Comparison of rates of glucose and acetoacetate removal by the isolated perfused rat brain with those measured in vivo

<table>
<thead>
<tr>
<th>Metabolic process</th>
<th>Perfused brain</th>
<th>Brain in vivo</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glucose removal (initial glucose concn. 5 mmol/litre)</td>
<td>0.78 ± 0.15 (5)</td>
<td>0.51</td>
<td>Hawkins et al. (1971)</td>
</tr>
<tr>
<td>Acetoacetate removal (initial concn. 1 mmol/litre)</td>
<td>0.14 ± 0.02 (4)</td>
<td>0.08</td>
<td>Hawkins et al. (1971)</td>
</tr>
</tbody>
</table>
Table 2. Concentrations of 5-hydroxytryptamine, dopamine, noradrenaline, L-tryptophan and L-tyrosine in rat brains during perfusion with a basal medium

Brains from well-fed male rats were perfused by using the method of Woods et al. (1974) with a basal medium containing glucose (10mmol/litre). After perfusion for various times the brains were rapidly removed for analysis. The control (unperfused) brains were rapidly removed after cervical dislocation. The values are µg/g wet wt. and are means ± S.E.M., with the numbers of observations in parentheses.

<table>
<thead>
<tr>
<th>Monoamine</th>
<th>Time (min)</th>
<th>Conc. in brain (µg/g wet wt.)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>0</td>
</tr>
<tr>
<td>5-Hydroxytryptamine</td>
<td>0.53±0.02 (4)</td>
<td>0.59±0.04 (4)</td>
</tr>
<tr>
<td>Dopamine</td>
<td>1.88±0.13 (3)</td>
<td>1.63±0.39 (3)</td>
</tr>
<tr>
<td>Noradrenaline</td>
<td>0.35±0.02 (3)</td>
<td>0.27±0.04 (3)</td>
</tr>
<tr>
<td>L-Tryptophan</td>
<td>2.91±0.71 (3)</td>
<td>3.99±0.93 (4)</td>
</tr>
<tr>
<td>L-Tyrosine</td>
<td>11.3±0.91 (4)</td>
<td>18.7±3.39 (3)</td>
</tr>
</tbody>
</table>

Monoamines and amino acids were determined at the end of perfusion. The basal medium contained, in addition to the constituents listed under 'Methods', glucose (10mmol/litre) and small quantities of amino acids. In particular, the concentrations (means ± S.E.M.) of tryptophan, tyrosine and phenylalanine were 0.95±0.01 (4), 2.35±0.72 (5) and 0.13±0.08 (5) µg/ml respectively (numbers of observations in parentheses).

The tryptophan concentration was maintained at the control value throughout the 2h perfusion, whereas that of tyrosine increased. This increase probably reflects uptake of tyrosine from the medium, and similar results have been reported by Krieglstein & Niemeyer (1975). The concentrations of 5-hydroxytryptamine, dopamine and noradrenaline were maintained at values close to those of control (unperfused) brains for the 2h perfusion (Table 2).

(c) 5-Hydroxytryptamine synthesis in perfused brain. We reported that the addition of the monoamine oxidase inhibitor, tranylcypromine (1 mmol/litre), to the medium caused an increase in brain 5-hydroxytryptamine content during the second hour of the perfusion at a mean rate of 0.16 µg/h per g (Graham et al., 1975). When tryptophan was added together with tranylcypromine, the rate of 5-hydroxytryptamine accumulation depended on the initial tryptophan concentration, being 0.16 µg/h per g with 0.1 mmol of tryptophan/litre and 0.4 µg/h per g with 1.0 mmol/litre, and the accumulation of 5-hydroxytryptamine continued throughout the perfusion. During perfusion with added tryptophan the content of this amino acid in brain rose progressively, reaching 14.7 µg/g after 2h when the initial tryptophan concentration was 0.1 mmol/litre and 63 µg/g when the initial concentration was 1 mmol/litre.

These results can be compared with those obtained after the administration of tranylcypromine and tryptophan to rats in vivo (Green & Youdim, 1975). These workers injected rats with tranylcypromine (20 mg/kg intraperitoneally (i.p.)) and 30min later administered tryptophan (100 mg/kg, i.p.). In their experiments the brain tryptophan content rose by 47.0 µg/g during the first hour and in the perfused brain by 40.3 µg/g. In these experiments in vivo the brain tryptophan concentration fell in the second hour owing to extracranial metabolism of tryptophan, whereas in the perfused brain it continued to rise, reaching 63 µg/g at 2h. After tryptophan loading in vivo (Green & Youdim, 1975) the mean rate of 5-hydroxytryptamine accumulation was 0.49 µg/h per g, which is very similar to that recorded above for the perfused brain (0.40 µg/h per g).

(d) Effect of phenylalanine on tryptophan accumulation and 5-hydroxytryptamine synthesis. The uptake of tryptophan into brain is dependent both on the concentration of tryptophan and on that of other amino acids in plasma (Fernstrom & Wurtman, 1972), since studies in vitro showed that other amino acids compete for uptake and efflux (Grahame-Smith & Parfitt, 1970; Parfitt & Grahame-Smith, 1974; McKean et al., 1976).
Table 3. Effect of L-phenylalanine on L-tryptophan accumulation and 5-hydroxytryptamine synthesis in perfused rat brain

Brains from well-fed male rats were perfused in all cases with a medium containing glucose (10 mmol/litre) and tranylcypromine (1 mmol/litre) to which was added either L-tryptophan (1 mmol/litre) alone or L-tryptophan (1 mmol/litre) and L-phenylalanine (1 mmol/litre). Perfusions were continued for 1 h. The values are means ± S.E.M., with the numbers of observations in parentheses. * P < 0.01, significantly different from the L-tryptophan content when L-tryptophan alone was present; † P < 0.02, significantly different from 5-hydroxytryptamine content when L-tryptophan alone was present.

<table>
<thead>
<tr>
<th>Additions to medium</th>
<th>L-Tryptophan (µg/g wet wt.)</th>
<th>L-Phenylalanine (µg/g wet wt.)</th>
<th>5-Hydroxytryptamine (µg/g wet wt.)</th>
</tr>
</thead>
<tbody>
<tr>
<td>None (brains not perfused)</td>
<td>2.91 ± 0.71 (4)</td>
<td>12.13 ± 1.68 (4)</td>
<td>0.53 ± 0.02 (4)</td>
</tr>
<tr>
<td>Tryptophan + tranylcypromine</td>
<td>43.3 ± 1.72 (3)</td>
<td>—</td>
<td>0.83 ± 0.02 (3)</td>
</tr>
<tr>
<td>Tryptophan + phenylalanine + tranylcypromine</td>
<td>29.6 ± 2.54 (3)*</td>
<td>50.3 (2)</td>
<td>0.63 ± 0.06 (3)†</td>
</tr>
</tbody>
</table>

1968). The perfused brain provides an experimental system in which it is possible to test whether, in intact tissue, other amino acids influence tryptophan uptake and 5-hydroxytryptamine synthesis.

Table 3 shows the effect of phenylalanine on tryptophan uptake and 5-hydroxytryptamine synthesis. When tranylcypromine and tryptophan are added to the medium at a concentration of 1 mmol/litre, there is an accumulation of tryptophan which is accompanied by a rise in 5-hydroxytryptamine concentration from 0.53 µg/g to 0.83 µg/g at 1 h. The addition of phenylalanine (1 mmol/litre) together with tryptophan (1 mmol/litre) and tranylcypromine (1 mmol/litre) resulted in a smaller uptake of tryptophan and a smaller synthesis of 5-hydroxytryptamine (Table 3).

General discussion

The purpose of the experiments described in the present paper was to ascertain whether the properties of the isolated perfused brain were comparable with the brain in vivo so far as monoamine metabolism is concerned.

The fact that the monoamine contents in brains perfused with the basal medium alone are maintained at control values suggests that the low concentrations of the amino acid precursors present in the medium or endogenous amino acids are sufficient to sustain the amine contents. In addition, these results also suggest that the process of perfusion does not markedly alter the metabolism of the amines at their sites of synthesis and storage.

As we have pointed out previously (Graham et al., 1975) with regard to 5-hydroxytryptamine synthesis, the properties of perfused brain closely resemble those found in rat brain in vivo. The experiments reported in the present paper concerning the effect of L-phenylalanine on L-tryptophan uptake and 5-hydroxytryptamine synthesis demonstrate the way in which the degree of control over the experimental conditions can be exploited to examine, in isolation, the effects of adding one amino acid on these processes.

We acknowledge the technical assistance of Mr. C. W. Graham and Miss Ann Marston.


Vol. 4
Less than a decade ago it was accepted biochemical dogma that the only energy-yielding substrate utilized by the brain was glucose. This belief was based on (a) the impairment of cerebral function in hypoglycaemic states, (b) the failure of potential energy-yielding substrates other than glucose to overcome the deleterious effects of hypoglycaemia and (c) measurements of arteriovenous differences across the head which indicated that glucose was the only circulating substrate taken up by the brain. Experiments which necessitated a reappraisal of the accepted view that the brain had an obligatory requirement for glucose were reported in 1967 by Cahill and his collaborators (Owen et al., 1967; Cahill et al., 1968). These experiments stemmed from calculations which suggested that in prolonged starvation sufficient glucose could not be synthesized from endogenous precursors to supply the metabolic needs of the brain and therefore other substrates must be utilized by this tissue. Measurements of arteriovenous differences in obese humans undergoing therapeutic starvation showed that the alternative substrates were ketone bodies, acetoacetate and 3-hydroxybutyrate (Owen et al., 1967). In this situation, ketone-body utilization accounted for about 60% of the oxygen consumed by the brain. This elegant experiment, with its teleological approach, renewed interest in the question of substrate supply to the brain and indicated the value of measurements of arteriovenous differences in such studies. An interesting question is why was this discovery not made earlier, because the technique of blood sampling from the internal jugular vein and an arterial site (femoral) was by no means new. At least two factors may have influenced the timing of the discovery, one being the somewhat earlier formulation of the role of free fatty acids as alternative substrates to glucose in many tissues (Fredrickson & Gordon, 1958) and the other the development of sensitive enzymic methods for the measurement of ketone bodies (Williamson et al., 1962).

The discovery that ketone bodies could be utilized by brain in prolonged starvation has stimulated further work on the question whether this is due to an adaptive change within the brain or simply to the increased availability of circulating ketone bodies.

One approach to this question is to measure the activities of the enzymes of ketone-body utilization in brains from animals in various physiological states. The results of such measurements in rat brain indicate that, in the adult, starvation, high-fat diets or