Br. J. Pharmac. (1976), **56**, 443–447

UPTAKE OF 5-HYDROXYTRYPTAMINE IN DIFFERENT PARTS OF THE BRAIN OF THE RABBIT AFTER INTRAVENTRICULAR INJECTION

R.C. DOW & I. LASZLO

MRC Brain Metabolism Unit, Department of Pharmacology, University of Edinburgh, 1, George Square, Edinburgh, EH8 9JZ

1 The uptake of 5-hydroxytryptamine (5-HT) was investigated in different areas of the rabbit brain (anterior hypothalamus, the raphe, the region of the substantia nigra, several cortical areas and the medulla oblongata) after intraventricular injection in pargyline pretreated animals by the formaldehyde-induced histochemical fluorescence method.

2 The distribution of fluorescence showed that the uptake of 5-HT, after circulation in the cerebrospinal fluid, caused a general increase in intensity of green yellow to yellow background fluorescence. There was an increased fluorescence in the nerve terminals, but no uptake occurred either in the cell bodies of neurones or in the glial cells.

Introduction

During an investigation of the uptake of 5-hydroxytryptamine (5-HT) after intraventricular injection, an accumulation of 5-HT was found in the glial cells of the area postrema of the rabbit (Dow, Laszlo & Ritchie, 1973) by the formaldehyde-induced fluorescence method described by Falck & Owman (1965). Biochemical estimations in the same experiments also showed an increase of 5-HT concentration in different areas of the brain. It appeared interesting to study the cellular distribution of 5-HT in other areas of the brain in order to find out if it is a general characteristic of the glial cells in the brain to take up this monoamine.

In this study the distribution of 5-HT in different areas of the brain was examined by the histochemical fluorescence method after intraventricular injection.

Methods

Thirty-three rabbits (New Zealand, white, of either sex, weight between 2.5 and 3.4 kg) were used.

Treatment with drugs: different doses of 5-HT creatinine sulphate (20, 40, 100 μg base) were injected into the right lateral ventricle through a guide tube by the method of Moir & Dow (1970) in 0.1 ml 0.9% w/v NaCl solution (saline). Pargyline hydrochloride (Eutonyl) 200 mg/kg was injected intraperitoneally alone, or 5 to 6 h before the injection of 5-HT. In each experiment one rabbit was used as a control without drugs, and one for obtaining tissue samples without formaldehyde treatment for the non-specific fluorescence. The rabbits in the control groups did not have the guide tubes implanted, since it was found that the intraventricular injection of saline did not influence the fluorescence picture. Animals were killed by injecting air into an ear vein 30 min after 5-HT injection. Brains were quickly removed, and areas to be investigated were dissected at 0°C. Visualization of monoamines was performed by the method of Falck & Owman (1965). The procedure used in these experiments with fluorescence microscopy and microspectrophotofluorimetry was described by Laszlo (1974). The fluorescence of the sections was observed with a BG3 (Schott) primary and a 50 (Zeiss) secondary filter. Various shades of colours are described by the nomenclature used by Loveland (1970), so that Green fluorescence corresponds to a wavelength of 513, and Yellow to 577, whilst in between Green-Yellow is 560, yellowish Green is 545 and greenish Yellow 570 nm. Identification of the areas was achieved by toluidine blue staining of the adjacent sections with the help of the stereotaxic atlas of the brain of the rabbit (Monnier & Gangloff, 1961).

In the preliminary experiments for the location of fluorescence, Nissl staining by the gallocyanine chromalum method (Drury & Wallington, 1967) was used on the same section as prepared for the fluorescence by the procedure described by Falck & Owman (1965). While this method had been found to be satisfactory for the study of the cellular localization of fluorescence in the area postrema (Fuxe & Owman, 1965; Dow *et al.*, 1973), in other parts of the brain it gave variable or uncertain results. Similar findings were obtained by using thionin and cresyl fast violet (Drury & Wallington, 1967) and Giemsa staining (Gurr, 1956). The reason for these discrepancies was

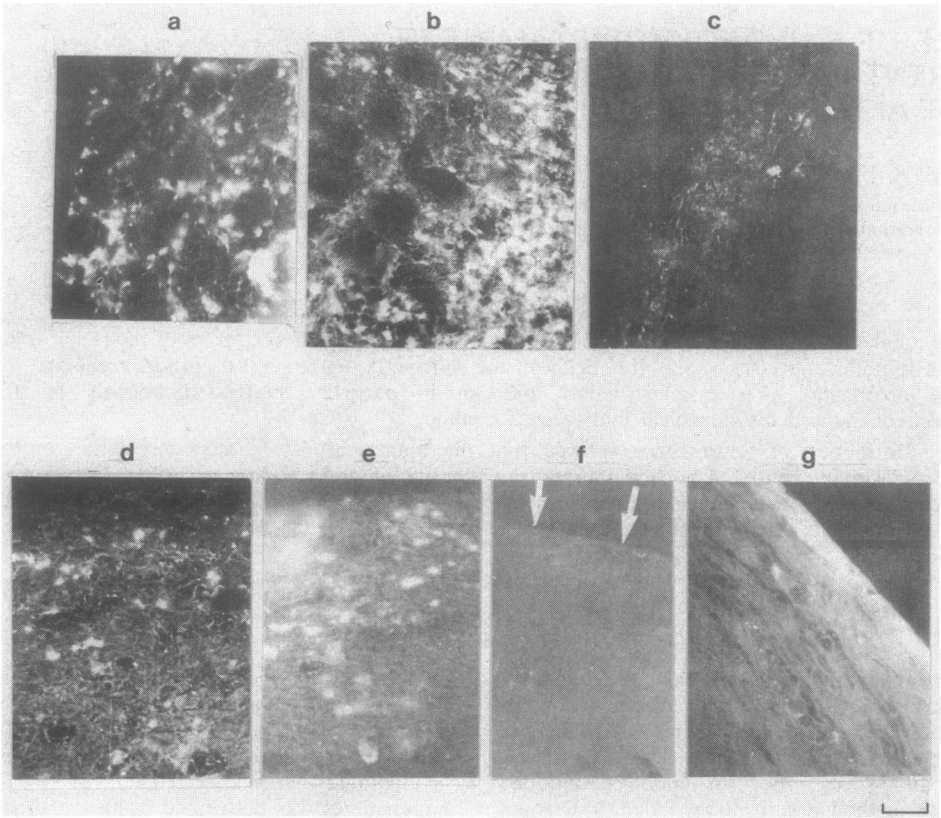

Figure 1 Fluorescence in various areas of the rabbit brain in control animals and after the intraventricular injection of various amounts of 5-hydroxytryptamine creatinine sulphate (5-HT) in animals given pargyline hydrochloride 5–6 h previously. (a) Nucleus supraopticus: control; (b) nucleus supraopticus: pargyline, 5-HT (100 μg); (c) nucleus suprachiasmaticus: pargyline, 5-HT (40 μg); (d) lateral wall of the third ventricle: control; (e) lateral wall of the third ventricle: pargyline, 5-HT (100 μg); (f) lateral surface of the hypothalamus: control (arrows indicate the surface of the hypothalamus); (g) lateral surface of the hypothalamus: pargyline, 5-HT (40 μg). Calibration 20 μm.

thought to be the difference between the procedures used for preparing sections for fluorescence microscopy (Falck & Owman, 1965) and those which are used for Nissl staining (Drury & Wallington, 1967). An attempt was made to adopt the procedure for Nissl staining described by Drury & Wallington (1967) on the sections prepared for fluorescence microscopy by the method of Falck & Owman (1965), but the quality of staining did not improve essentially. Methods for staining glial cells, e.g. by Cajal's gold chloride sublimate method for astrocytes, Hortega's silver carbonate method for astrocytes, and Hortega's silver carbonate method for microglia (Drury & Wallington, 1967) were also tried on different animals, but none of these methods appeared to be applicable for sections prepared for fluorescence microscopy.

Since sections after fluorescence microscopy cannot be stained properly by any of the above mentioned methods, the cellular location of fluorescence was based on sections stained from different animals by the procedure described for Nissl staining by the gallocyanin chromalum method (Drury & Wallington, 1967).

Fluorescent sections were photographed on Ilford FP4, Din 22, with an exposure time from 45 to 60 s, or Ilford HP4 black and white films (Din 27–29) 15 to 60 s exposures.

Results

The fluorescence in the sections from the animals treated with different doses of 5-HT (20, 40 and

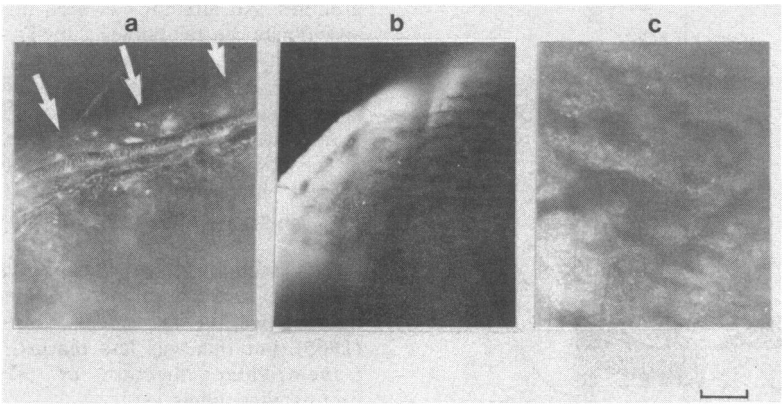

Figure 2 Fluorescence in various areas of the rabbit brain in control animals and after the intraventricular injection of various amounts of 5-hydroxytryptamine creatinine sulphate (5-HT) in animals given pargyline hydrochloride 5–6 h previously. (a) Cortex (area postcentralis): control (arrows indicate the surface of the cortex); (b) cortex (area postcentralis): pargyline, 5-HT (40 μg); (c) cortex (area postcentralis): pargyline, 5-HT (40 μg). Calibration 20 μm.

100 μg) was compared to that of the untreated (control) animals, and the following results were found.

Anterior hypothalamus: the Green background fluorescence of this area, including the chiasma opticum and the tractus opticus was more intense after 20 μg of 5-HT than in the control animals; after 40 and 100 μg, this difference was more marked, and the sections showed Green-Yellow fluorescence. The nerve endings in the whole area, especially in the nucleus supraopticus exhibited increased intensity of Green or Green-Yellow fluorescence after 20 μg of 5-HT. The nerve endings also appeared to be present in larger numbers, and the preterminals appeared elongated after 20 μg of 5-HT; after 40 and 100 μg, these differences were even more pronounced. In spite of the increased intensity of the fluorescence in the whole area, no uptake of 5-HT by cell bodies of neurones or glial cells was found (Figure 1a,b,c).

The ependyma of the third ventricle did not show fluorescence in either the sections from the control, or from the 5-HT-treated animals, although the area around the third ventricle exhibited increased intensity of fluorescence (Figure 1d,e) The surface of the brain, including the optic chiasma, did not exhibit fluorescence in control animals, but in the 5-HT-treated animals (20 μg) it showed increased intensity of yellowish Green fluorescence, which after higher doses (40 μg) of 5-HT became a fairly intense Green-Yellow or greenish-Yellow diffuse fluorescence layer (Figure 1f,g; Figure 2a,b,c). After 100 μg the colour in some cases showed Yellow, quickly fading fluorescence, as was found at the surface of the optic chiasma and in the meninges. The wall of the blood vessels, lateral to the nucleus supraopticus, after 100 μg of 5-HT also exhibited intense Yellow fluorescence. Estimates of intensity by microspectrophotofluorimetric method showed a similar distribution of fluorescence intensity to that described above.

The fluorescence picture in other parts of the brain, e.g. in the substantia nigra and in adjacent areas, in the raphe nuclei and in cortical areas as described by Falck, Mchedlishvili & Owman (1965), (e.g. the area postcentralis and insularis, the area occipitalis and temporalis, and the area occipitotemporalis) showed an essentially similar distribution of fluorescence. In these areas the background fluorescence after 20 μg of 5-HT (and pargyline) usually exhibited more intense Green or yellowish Green fluorescence than sections from control animals; after 40 μg the fluorescence became more intense Green-Yellow, and after 100 μg a greenish Yellow to Yellow very intense diffuse fluorescence was usually found. In spite of the generally increased intensity of background fluorescence, the cells remained dark in the whole area (Figure 2a,b,c).

After 5-HT-treatment the Green fluorescence, which is normally seen only at the terminals of nerve fibres in control animals, spread along the fibre so that they appeared more elongated. There was also more intense Green-Yellow fluorescence in the brain as well as around blood vessels at the surface of the brain.

The ependyma of the lateral ventricles and of the aqueduct in some cases after 5-HT showed a very quickly fading Yellow or Green fluorescent layer. In pargyline pretreated animals the surface of the brain and the areas near to it, after 20 μg of 5-HT exhibited

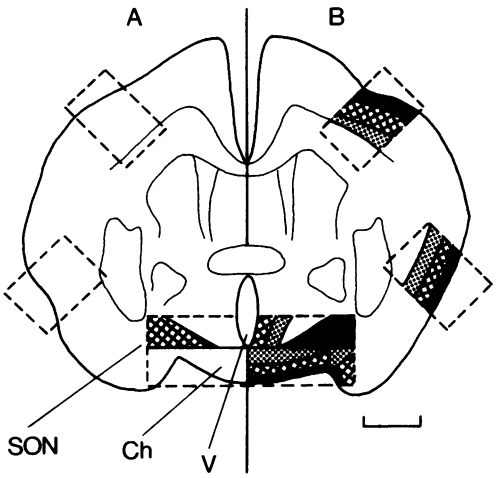

Figure 3 Schematic representation of the uptake of 5-hydroxytryptamine in different areas of the rabbit brain after intraventricular injection. Frontal plane corresponding to the level of the area preoptica (redrawn after Monnier & Gangloff, 1961). SON = supraoptic nucleus; Ch = optic chiasm; V = third ventricle; A = control; B = 40 or 100 μg 5-hydroxytryptamine creatinine sulphate 5–6 h after pargyline. Calibration: 3 mm. Areas marked by---line indicate the regions investigated. Intensity of fluorescence: dotted areas = low; cross-hatched areas = medium; shaded areas = high intensity.

Green or Green-Yellow diffuse fluorescence, and an intense fluorescent layer was usually present, corresponding to the meninges; after 40 and 100 μg the colour of fluorescence became greenish Yellow or Yellow. The fluorescence at the surface after the photographic exposure (1 min) became Green. After the intraventricular injection of 5-HT, a layer exhibiting an increased fluorescence, compared to the control, was found 1 to 2 mm from the external and internal surfaces of the brain (Figure 1d,e; Figure 2a,b). A schematic representation of the uptake of 5-HT can be seen in Figure 3 in the plane corresponding to the anterior hypothalamus. Similar distribution of the uptake was found in the region corresponding to the frontal planes of the raphe nuclei and the substantia nigra as well. Walls of blood vessels (at the surface of the brain) after 5-HT-treatment exhibited increased intensity of Green-Yellow fluorescence.

The fluorescence of the area postrema after 100 μg of 5-HT and pargyline was examined, and was found to be similar to that which had been described by Dow *et al.* (1973), showing increased number and intensity of Green fluorescent cells and Yellow fluorescent granules. No attempt has been made to characterize specifically the cell bodies showing fluorescence in the area postrema; it is probable from our earlier studies (Dow *et al.*, 1973) that they are glial cells. The ependyma of the central canal exhibited a very quickly fading fluorescent layer, which was more intense than in the control. Adjacent to the area postrema were present increased number of Green fluorescent varicosities, but there was no uptake of 5-HT by cell bodies.

The sections from animals injected only with pargyline showed an increased intensity of specific fluorescence, in agreement with Fuxe & Owman (1965), but this was less marked than after the intraventricular injection of 5-HT in pargyline pretreated animals.

Discussion

The earlier finding that glial cells in the area postrema of the rabbit contained 5-HT after intraventricular injection (Dow *et al.*, 1973) prompted a study of the uptake in other parts of the brain under the same experimental conditions. The areas of this study were selected on functional as well as morphological grounds, e.g. the anterior hypothalamus and the region of the raphe nuclei, which contained 5-HT nerve terminals and nerve cells, respectively (Carlsson, Falck & Hillarp, 1962; Fuxe, Hökfelt & Ungerstedt, 1969; Fuxe & Jonsson, 1974; Jonsson, Einarsson, Fuxe & Hallman, 1974); the cortical areas and the region of the substantia nigra, where the cell bodies of neurones and glial cells could be identified on the basis of morphological differences. This was necessary since identification by the conventional staining methods (e.g. Nissl staining or glial staining) on the sections for fluorescence microscopy did not appear satisfactory. The presence of freeze artefacts in cell bodies or freeze dried sections were mentioned by Dahlström & Fuxe (1964), who found that Nissl staining did not give satisfactory results on these sections.

The region adjacent to the area postrema also appeared interesting for this study, since the uptake of 5-HT by glial cells had been found previously in the area postrema (Dow *et al.*, 1973). The results obtained by the histochemical fluorescence method showed no uptake of 5-HT by cell bodies of neurones or glial cells in any of these parts of the brain, apart from the area postrema, which had been described earlier (Dow *et al.*, 1973). In contrast nerve endings, especially in the anterior hypothalamus, showed a marked uptake of this monoamine. An intense fluorescence was regularly found in the wall of the blood vessels after 5-HT treatment, as in the previous study by Dow *et al.* (1973). An accumulation of noradrenaline and 5-HT in arterial smooth muscle was also described by Buchan, Lewis & Sugrue (1974).

The colour of fluorescence after increasing doses of 5-HT changed toward the longer wavelengths; a shift of colour during photographic exposure in the opposite direction was also found in sections of 5-HT-treated animals. Both phenomena can be explained by the relationship between concentration and colour of fluorescence described by Laszlo (1975a, 1975b). The quickly fading fluorescence in the sections is characteristic of 5-HT (Falck & Owman, 1965).

The lack of uptake of 5-HT by the glial cells in different parts of the brain compared to the area postrema after its intraventricular injection can be explained by the characteristics of glial cells and those of the ependyma in these areas. The flat cell bodies of the ependyma over the area postrema differ from those of the rest of the brain (Wislocki & Putnam, 1920; Clemente & Breemen, 1955; Morest, 1960), and a free access of 5-HT could occur from the cerebrospinal fluid, where it is present in a high concentration after intraventricular injection. The glial cells in the area postrema also differ structurally from mature astrocytes in other parts of the brain (Wislocki & Putnam, 1920; Clemente & Breemen, 1955; Morest, 1960).

The uptake of 5-HT after intraventricular injection in pargyline pretreated rabbits produces greater intensity of fluorescence from both surfaces of the brain inward. Neither cell bodies of neurones nor glial cells appeared to contain this monoamine other than in the area postrema.

We are very grateful to Dr G.W. Ashcroft and to Dr D. Eccleston for the continuous help and discussion during this study, and to Dr G. Arbuthnott for his help in the preparation of the manuscript. We wish also to thank Mr I. Lennox for the illustration, and Abbot Laboratories Ltd., for the generous supply of pargyline hydrochloride (Eutonyl).

References

BUCHAN, P., LEWIS, A.J. & SUGRUE, M.F. (1974). A comparison of the accumulation of noradrenaline and 5-hydroxytryptamine into arterial smooth muscle. *Br. J. Pharmac.*, **52**, 132–133P.

CARLSSON, A., FALCK, B. & HILLARP, N.-Å. (1962). Cellular localization of brain monoamines. *Acta physiol. scand.*, **56**, Suppl. 196.

CLEMENTE, C.D. & VAN BREEMEN, V.L. (1955). Nerve fibers in the area postrema of cat, rabbit, guinea pig and rat. *Anat. Rec.*, **123**, 65–79.

DAHLSTRÖM, A. & FUXE, K. (1964). Evidence for the existence of monoamine-containing neurons in the central nervous system. I. Demonstration of monoamines in the cell bodies of brain stem neurons. *Acta physiol. scand.*, **62**, Suppl. *232*.

DOW, R.C., LASZLO, I. & RITCHIE, I.M. (1973). Cellular localization of the uptake of 5-hydroxytryptamine in the area postrema of the rabbit after injection into a lateral ventricle. *Br. J. Pharmac.*, **49**, 580–587.

DRURY, R.A.B. & WALLINGTON, E.A. (1967). *Carleton's Histological Technique.* 4th edition. pp. 266–280. New York, Toronto: Oxford University Press.

FALCK, B., MCHEDLISHVILI, G.I. & OWMAN, Ch. (1965). Histochemical demonstration of adrenergic nerves in cortex-pia of rabbit. *Acta pharmac. tox.*, **23**, 133–142.

FALCK, B. & OWMAN, C. (1965). A detailed methodological description of the method for the cellular demonstration of biogenic monoamines. *Acta Univ. lund.* Sect. II. No. **7**, 1–23.

FUXE, K., HÖKFELT, T. & UNGERSTEDT, U. (1969). Distribution of monoamines in the mammalian central nervous system by histochemical studies. In *Metabolism of Amines in the Brain.* Proceedings of a Symposium of the British and Scandinavian Pharmacological Societies. Edinburgh, July, 1968. ed. Hooper, G. pp. 10–22. London: McMillan and Co. Ltd.

FUXE, K. & JONSSON, G. (1974). Further mapping of central hydroxytryptamine neurons: studies with the neurotoxic dihydroxytryptamines. In *Advances in Biochemical Psychopharmacology.* Vol. 10, ed. Costa, E. & Greengard, P. pp. 1–12. New York: Raven Press.

FUXE, K. & OWMAN, C. (1965). Cellular localization of monoamines in the area postrema of certain mammals. *J. comp. Neurol.*, **125**, 337–354.

GURR, E. (1956). *A Practical Manual of Medical and Biological Staining Techniques,* p. 106. London: Leonard Hill (Books) Ltd.

JONSSON, G., EINARSSON, P., FUXE, K. & HALLMAN, H. (1974). Microspectrofluorimetric studies on central 5-hydroxytryptamine neurons. In *Advances in Biochemical Psychopharmacology.* Vol. 10, ed. Costa, E & Greengard, P. pp. 55–65. New York: Raven Press.

LASZLO, I. (1974). Procedure for the histochemical demonstration of noradrenaline and 5-hydroxytryptamine in the central nervous system using the formaldehyde induced fluorescence method. *Lab. Pract.*, **23**, 299–304.

LASZLO, I. (1975a). Relationship between colour and concentration of noradrenaline and 5-hydroxytryptamine in the formaldehyde induced fluorescence method. *Lab. Pract.*, **24**, 146–150.

LASZLO, I. (1975b). Change of colour of fluorescence during exposure to ultraviolet light and its effect on photomicrography. *Med. biol. Illust.*, **25**, 97–102.

LOVELAND, R.P. (1970). *Photomicrography.* vol. 1, p. 3. New York: John Wiley and Sons, Inc.

MOIR, A.T.B. & DOW, R.C. (1970). A simple method for allowing perfusion of cerebral ventricles of the conscious rabbit. *J. appl. Physiol.*, **28**, 528–529.

MONNIER, M. & GANGLOFF, H. (1961). *Rabbit Brain Research.* Vol. 1. Atlas for Stereotaxic Brain Research on the Conscious Rabbit. Amsterdam: Elsevier.

MOREST, D.K. (1960). A study of the structure of the area postrema with Golgi methods. *Am. J. Anat.*, **107**, 291–303.

WISLOCKI, G.B. & PUTNAM, T.J. (1920). Note on the anatomy of the areae postremae. *Anat. Rec.*, **19**, 281–287.

(Received September 17, 1975. Revised December 22, 1975.)