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Cellular localization of the uptake of 5-hydroxytryptamine in the area postrema of the rabbit after injection into a lateral ventricle

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Summary

1. The cellular localization of 5-hydroxytryptamine (5-HT) was investigated in the area postrema of the rabbit after intraventricular injection under different experimental conditions.

2. 5-HT was found to be acccumulated in different parts in and near to the area postrema, e.g. in glial cells, dorsal surface of the area postrema and ependyma of the central canal.

3. The concentrations of 5-HT and 5-hydroxy indol-3-yl acetic acid (5-HIAA) were measured in different parts of the brain and CSF in control and in 5-HT treated animals with and without pargyline pretreatment. Intraventricular injection of 5-HT increased the concentration of 5-HT and 5-HIAA in the brain and in the CSF; pretreatment with pargyline further increased the concentrations of 5-HT but decreased the concentration of 5-HIAA.

Introduction

The localization of the uptake of 5-hydroxytryptamine (5-HT) after intraventricular injection was investigated in the area postrema of the rabbit. This region was chosen because of its anatomical situation at one of the exits from the fourth ventricle, and may subserve the purpose of removal of acids or amines from the cerebrospinal fluid (CSF). Its close proximity to the choroid plexus of the fourth ventricle, which is known to have the capacity to remove 5-hydroxy indol-3-yl acetic acid (5-HIAA) (Pullar, 1971), also emphasized this possibility. These results have been communicated to the British Pharmacological Society. (Dow, Laszlo & Ritchie, 1972).

Methods

Forty-four rabbits (New Zealand, white) of either sex (average weight between 2·5 and 3·5 kg) were used.

Treatment with drugs. Different doses of 5-HT creatinine sulphate were injected into the right lateral ventricle through a guide tube inserted into the skull by the method of Moir & Dow (1970). Doses of 5-HT, calculated as base, were injected in 0·1 ml 0·9% w/v NaCl solution (saline), or in some experiments in artificial cerebrospinal fluid (Moir & Dow, 1970). Control animals were usually injected with the same volume of saline or artificial CSF.

In some experiments pargyline hydrochloride (Eutonyl), 200 mg/kg, was injected intraperitoneally, usually 5 to 6 h before 5-HT, and in others pargyline was administered intraperitoneally without subsequent 5-HT treatment. Control animals were treated with an intraperitoneal injection of saline. Animals were killed by air injection into the ear vein at varying intervals after 5-HT injections. Samples of CSF were taken through a guide tube inserted into the cisterna and also from the lateral ventricle. The guide tubes were usually inserted into the skull two weeks before the experiments.

Visualization of 5-HT and noradrenaline was essentially by the method described by Falck & Owman (1965), with certain modifications of the procedure (Laszlo, 1972). Localization of uptake of 5-HT was based on the comparison of fluorescence with Nissl staining (gallocyanin chromalum method ; Drury & Wallington, 1967) on the sections prepared for fluorescence microscopy (Falck & Owman, 1965).

In each experiment for any specific dose and time, control tissues of untreated animals were examined with and without formaldehyde treatment.

Estimation of 5-hydroxytryptamine and 5-hydroxy indol-3-yl acetic acid. 5-HT was assayed as described by Shields & Eccleston (1972), and 5-HIAA by the method of Eccleston, Moir, Reading & Ritchie (1966). The brain was homogenized in 0·4 N perchloric acid with subsequent precipitation of potassium perchlorate by adjusting pH to 8·4 with KOH. The supernatant was passed over a cation exchange column of Amberlite CG 50, and after washing the 5-HT was eluted off in 2 N sulphuric acid. It was subsequently estimated in a spectrophotofluorimeter by activation scan, after addition of conc. HCl ; fluorescence maximum 305 nm (activation) and 530 nm (fluorescence). The 5-HIAA was extracted from the first column effluent, after salt saturation and acidification, into diethyl ether. This was washed with salt saturated dilute HCl, and the 5-HIAA back extracted with 0·3 M borate buffer. The fluorescence was determined as for 5-HT.

Results

Fluorescence microscopy. The appearance of the area postrema of the control animals was found to be essentially the same as described by Fuxe & Owman (1965).

The effect of intraventricular 5-HT treatment was investigated (a) after different doses of 5-HT at a fixed interval (30 min), (b) in pargyline-pretreated animals after different doses of 5-HT from 10 min to 18 h intervals ; (c) the effect of an injection of pargyline alone was investigated. The effects of these treatments are described in the following paragraphs.

(a) 5-Hydroxytryptamine treatment (30 minutes)

5-HT, 20 μg (6 animals): This dose increased the fluorescence of the area postrema compared to that seen in the control animals, but the individual cells remained distinguishable, therefore this dose was chosen for further investigations. The effect of this dose at this time interval was: (a) an increase in the number and intensity of green fluorescent cells and yellow fluorescent granules ; (b) an increase in the background fluorescence (Fig. 1a and 1b). The intensity of fluorescence of both background and that of the green fluorescent cells was found by microspectrophotofluorimetric measurements to be increased by 20 to 50% ; (c) the development of green or yellow fluorescence of the dorsal surface of the

area postrema corresponding to the arachnoidea and pia mater (Fig. 1c and 1d); (d) an increase of yellow fluorescence of the ependyma of the central canal, not extending to the central surface of the area postrema (Figure 1e, 1f and 1g).

5-Hydroxytryptamine 200 μg (3 animals). After this dose the whole section exhibited an intense green fluorescence, and the fluorescent cells in the area postrema present in the control animals could not be distinguished, possibly due to the strong background fluorescence. This dose therefore appeared too high to investigate the cellular localization of 5-HT uptake. Other neighbouring areas (e.g. choroid plexus, cerebellum, wall of the basilar artery) also exhibited very intense fluorescence after this dose.

(b) Pargyline, 5-hydroxytryptamine (different doses and intervals)

5-HT, 5 μg 30 min; after pretreatment with pargyline (1 animal): A noticeable increase in the intensity of the green background fluorescence and in the number of green intensely fluorescent cells was found, compared to that of the control. A similar effect was also found 10 min after treatment with 20 μg of 5-HT in pargyline-pretreated animals.

5-HT, 20 μg, 30 min; after pretreatment with pargyline (6 animals): Essentially the same fluorescence was found as with 5-HT alone (after the same dose and time), but a generally increased intensity of fluorescence was found in the whole area postrema.

The effect of the same treatment was investigated after longer intervals; 2 h after the injection of 5-HT (1 animal), a further increase of fluorescence was found, compared to the effect after 30 minutes. Because of the increased intensity of background fluorescence it was not possible to discern individual cells. The whole section, including the area postrema and the choroid plexus showed strong fluorescence, i.e. the increased fluorescence was not restricted to the area postrema. The fluorescence was, in general, more intense, but less defined than in the animals in which the effect was investigated 30 min after 5-HT treatment. Although it was intended to investigate the effect of 5-HT after longer intervals, the animals died after this treatment (e.g. 3 h 20 min after the injection of 5-HT).

(c) Pargyline, 4 hours 45 minutes (1 animal)

Sections after pargyline treatment showed somewhat more intense green background fluorescence and there was an increased number of multipolar nerve cells, which exhibited yellow fluorescence in the cytoplasm, than in the control animals. Fuxe & Owman (1965) also found similar effect of pargyline on the fluorescence of the area postrema.

An increased number of green fluorescent cells in the 5-HT-treated animals was repeatedly found. The difference between the number of these cells in the two groups of animals was great; it was sometimes difficult to find green fluorescent cells (noradrenaline containing nerve cells described by Fuxe & Owman, 1965) in the section from control animals. In the 5-HT-treated animals many green fluorescent cells were present and it was difficult to find cell-free areas to measure the intensity of background fluorescence.

Localization of the uptake of 5-hydroxtryptamine. The localization of the uptake of 5-HT was investigated by Nissl staining on the same sections, after fluorescence

TABLE 1a. *Concentration of 5-hydroxytryptamine (5-HT) in the brain (ng/g) and in the CSF (ng/ml) after intraperitoneal injection of pargyline (200 mg per kg) and after intraventricular injection of 5-HT (20 μg) with and without pretreatment with pargyline (200 mg per kg, intraperitoneally).*

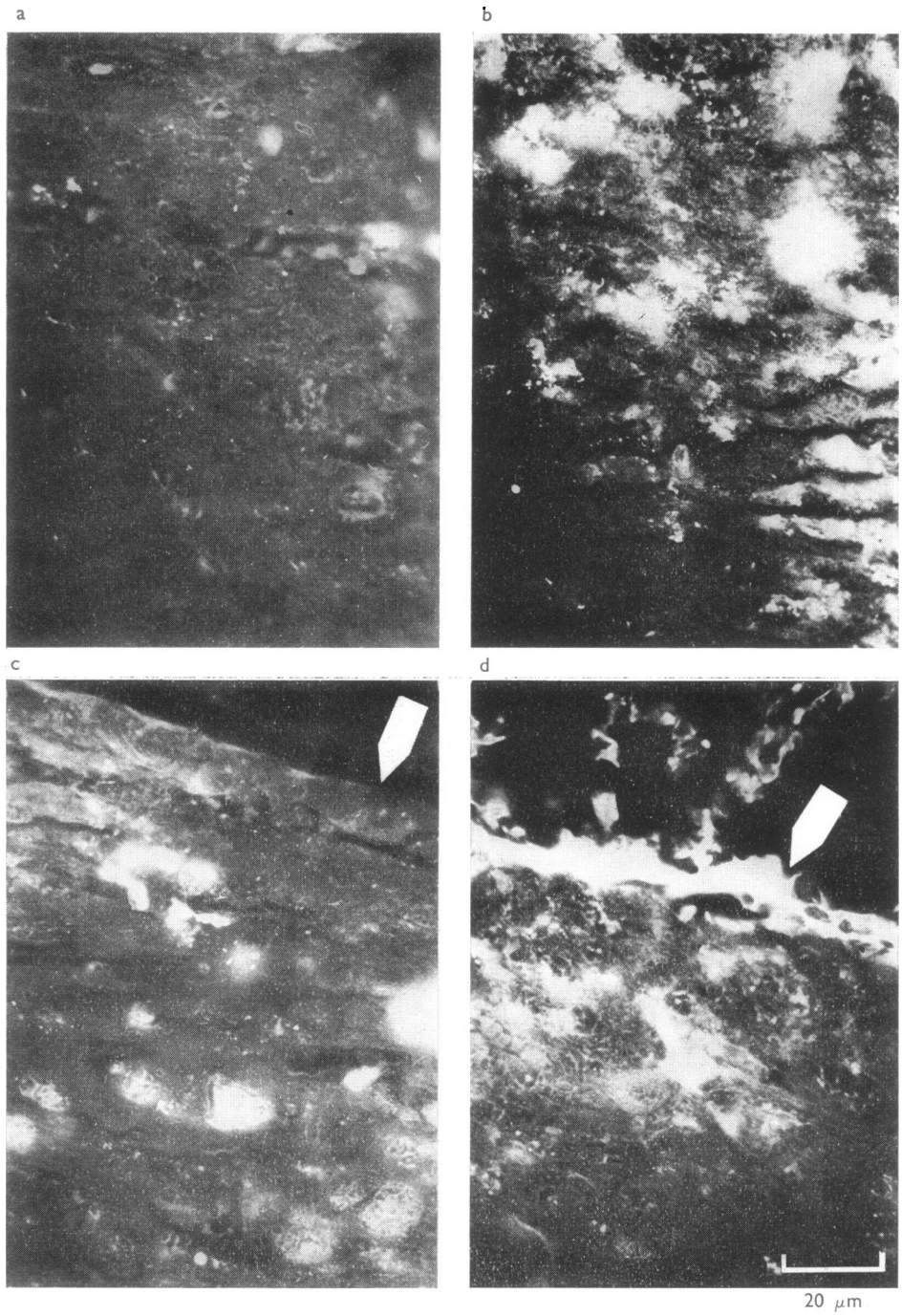
				Treatment				
				Pargyline (6 h), 5-HT (different intervals)				
	Area	Control†	5-HT (30 min)	Pargyline*	10 min	30 min	2 h	3 h 20 min
Brain	fore-brain	451±83 (7)	496 (1)	902±160 (5)	2013 (1)	1683±252 (3)	1166 (1)	1851 (1)
	mid-brain	694±135 (7)	974 (1)	1902±342 (5)	5735 (1)	3003±364 (3)	2605 (1)	3788 (1)
	hind-brain	805±87 (7)	2162 (1)	2002±463 (5)	2640 (1)	3464±1256 (3)	1800 (1)	4444 (1)
CSF	ventricular	0(<10) (4)				32 (1)		2923 (1)
	cisternal	0 (<10) (3)	851±182 (3)	72±83 (3)	4052 (1)	494 288 (2)	141 (1)	861 (1)

Values: mean ± standard deviation. ( ) = number of observations. * pooled data from animals treated with pargyline for 4 h 30 min, 4 h 38 min, 6 h 30 min and 8 hours; † values derive from control animals and from samples taken before 5-HT injection.

TABLE 1b. *Concentration of 5-hydroxyindol-3-yl acetic acid (5-HIAA) in the brain (ng/g) and in the CSF (ng/ml) after intraperitoneal injection of pargyline (200 mg per kg) and after intraventricular injection of 5-hydroxytryptamine (5-HT) with and without pretreatment with pargyline (200 mg per kg, intraperitoneally).*

				Treatment				
				Pargyline (6 h), 5-HT (different intervals)				
	Area	Control†	5-HT (30 min)	Pargyline*	10 min	30 min	2 h	3 h 20 min
Brain	fore-brain	329±101 (7)	545 (1)	125±71 (5)	65 (1)	188±115 (3)	58 (1)	54 (1)
	mid-brain	849±295 (7)	1422 (1)	357±199 (5)	174 (1)	529±156 (3)	149 (1)	403 (1)
	hind-brain	1058±288 (7)	828 (1)	333±212 (5)	165 (1)	531±185 (3)	127 (1)	297 (1)
CSF	ventricular	98±19 (4)				76 (1) 175		114 (1)
	cisternal	72±47 (6)	444±290 (4)	45±31 (5)	0 (<10) (1)	5 (2)	0 (<10) (1)	52 (1)

Valves: mean ± standard deviation. ( ) = number of observations. *Pooled data from animals treated with pargyline for 4 h 30 min, 4 h 38 min, 6 h 30 min and 8 hours; † values derive from control (untreated animals) and from samples before 5-HT injection.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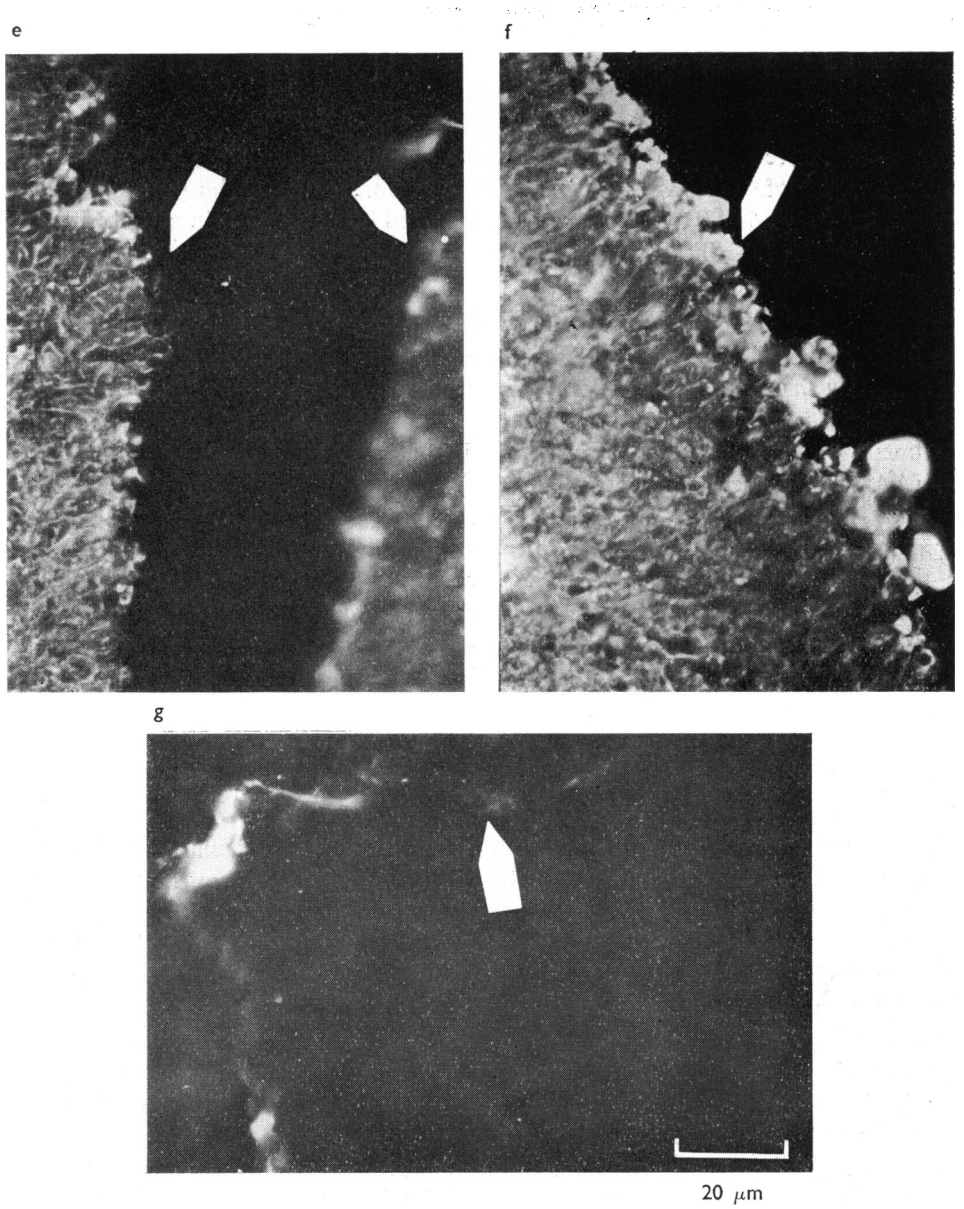

FIG. 1. (a): Control area postrema: few green, not intensely fluorescent cells; dark background. (b): Area postrema after 5-hydroxytryptamine treatment (20 μg, intraventricularly; 30 min): the number and intensity of green fluorescent cells and of yellow fluorescent granules is increased. More intense background fluorescence. (c): Control: the dorsal surface of the area postrema (→) does not show fluorescence. (d): Area postrema after 5-hydroxytryptamine treatment (20 μg, intraventricularly; 30 min): yellow fluorescence layer on the dorsal surface of the area postrema (→); Calibration mark=20 μm. (e): Control: ependyma of the fourth ventricle (→) shows only a faint yellow fluorescence. (f): Area postrema after 5-hydroxytryptamine treatment (20 μg, intraventricularly; 30 min): the ependyma of the fourth ventricle (→) exhibits an intense yellow fluorescence. (g): Area postrema after 5-hydroxytryptamine treatment (20 μg, intraventricularly; 30 min): the fluorescence of the ependyma does not extend to the ventral surface of the area postrema (→); Calibration mark=20 μm.

microscopy. It was found that in the control animals, of the large number of cells present in the area postrema, only a few cells exhibited fluorescence; these are the noradrenaline containing nerve cells described by Fuxe & Owman (1965). After pargyline and 5-HT treatment (20 μg, 30 min) cells exhibiting green fluorescence were identified on the basis of Nissl staining, as glial cells. In these sections, walls of blood vessels also exhibited fluorescence. The green colour of fluorescence after 5-HT treatment can be explained by the finding in model (droplet) experiments, that 5-HT in a certain range of concentrations can be green (Laszlo, 1972).

Chemical estimations. In some experiments the concentration of 5-HT and 5-HIAA in the CSF and in the brain were measured (Table 1a, and 1b). These results show that in control animals there was no 5-HT ($<$10 ng/ml) in the CSF; there was usually a measurable amount ($>$10 ng/ml) of 5-HIAA present. After treatment with pargyline 5-HT was found in the CSF and the concentration of 5-HIAA was reduced. The intraventricular injection of 5-HT caused an increase in both 5-HT and 5-HIAA in the CSF. When treatment with pargyline was combined with an intraventricular injection of 5-HT there was a further increase in the concentration of 5-HT but the concentration of 5-HIAA was lower than in controls. The highest concentration of 5-HT was observed 10 min after the intraventricular injection. These changes in the concentrations of 5-HT and 5-HIAA in the CSF were accompanied by parallel changes in the concentrations of these substances in the brain tissue.

Discussion

The localization of the uptake of 5-HT after intraventricular injection was investigated in the area postrema of the rabbit by the histochemical fluorescence method described by Falck & Owman (1965) with some modification of the procedure (Laszlo, 1972). Earlier, the uptake of 5-HT was investigated after intraventricular injection by Aghajanian, Bloom, Lovell, Sheard & Freedman (1966) by using tritiated 5-HT in rat, and the cellular localization was assessed by autoradiography. They found only a few grains within the glial cell bodies. Fuxe & Ungerstedt (1967) also used intraventricular injection for the study of localization of 5-HT, but uptake of 5-HT by glial cells was not mentioned. The lack of uptake of 5-HT by glial cells in the above mentioned reports can be accounted for by the different experimental conditions (e.g. species difference, region under investigation, dose, etc.). Recently Henn & Hamberger (1971) found the accumulation of 5-HT in a separated fraction of the brain containing glial cells, which supports the observations described in the present work.

In the present experiments the uptake of 5-HT was located, after fluorescence microscopy, by Nissl staining on the same section, as described by Falck & Owman (1965). Experimental conditions were investigated for the evaluation of the uptake of 5-HT by using different doses of 5-HT with or without pargyline treatment, and also different time intervals for the action of these drugs. Pargyline was used in the same dose and range of time interval, as described by Fuxe & Owman (1965). It appears that 30 min after a 20 μg dose of 5-HT in pargyline treated animals gave the optimal conditions for the evaluation of the increased fluorescence, when individual cells could still be distinguished.

Uptake of 5-HT occurs in the whole area postrema, in glial cells, ependyma of the central canal and dorsal surface of the area postrema. Pargyline pretreatment further increased the accumulation of 5-HT. The accumulation of 5-HT in non-neuronal structures, e.g. in glial cells may have significance in the mechanism by which this transmitter substance is eliminated at, or near the site of its release.

In the experiments described above, after treatment with 5-HT the area postrema exhibited increased intensity of fluorescence, the chemical estimations also showed a higher concentration of 5-HT in different parts of the brain and in the CSF. The effects of pargyline can also be seen in the results of the chemical estimations, e.g. the increased concentration of 5-HT, decreased concentration of 5-HIAA in the brain tissue, and the presence of 5-HT in the CSF.

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