Localization of fenfluramine and reserpine in brain regions of rats with extensive degeneration of 5-hydroxytryptaminergic neurons

T. MENNINI, R. PATACCINI, V. CRUNELLI, S. CACCIA, M. BALLABIO, R. SAMANIN, S. GARATTINI*, *Istituto di Ricerche Farmacologiche 'Mario Negri', Via Eritrea, 62 20157, Italy*

Several reports (see Garattini et al 1978, 1979 for references) indicate that fenfluramine exerts its anorectic activity by selectively affecting 5-hydroxytryptamine mechanisms in the brain. At doses markedly decreasing food intake in various animal species (Le Douarec & Schmitt 1964) it releases 5-HT from nerve terminals and inhibits its reuptake (Alphin & Ward 1969; Fuxe et al 1975; Kannengiesser et al 1976; Garattini et al 1978), but appears to have a limited effect on post-synaptic 5-HT receptors (Garattini et al 1979). Both the 5-HT depletion induced by fenfluramine and its anorectic activity are selectively inhibited by chlorimipramine a 5-HT-uptake inhibitor, which presumably prevents fenfluramine entering the 5-HT-ergic system in the brain (Ghezzi et al 1973). Electrolytic lesion of 5-HT-ergic neurons also counteracts fenfluramine-induced anorexia (Samanin et al 1972). Recently Belin et al (1976) reported a decrease [^{14}C]fenfluramine binding in the hypothalamus and mesencephalon of rats injected intracerebrally with 5,6-dihydroxytryptamine (5,6-HT), an agent causing degeneration of 5-HT neurons (Baumgarten et al 1971).

We have investigated whether the reported reduction in the anorectic effect of fenfluramine in rats with electrolytic lesions of 5-HT-ergic neurons could be related to lower drug concentrations in different brain areas. For comparison, we studied the effect of the same lesion on the localization of [^{3}H]reserpine in brain areas, through selective labelling of brain nerve terminals after its in vivo injection (Mennini et al 1977). This is an interesting 'model' for a critical approach to the study of irreversible drug binding to brain cellular and subcellular components (Manara et al 1974).

Female CD-COBS rats (200–225 g) (Charles River, Italy) with free access to food and housed at constant temperature (22°C) and relative humidity (60%) were used. Electrolytic lesions of the nucleus raphe dorsalis (DR) were produced by applying a 2·0 mA anodal current for 15 s through a stainless-steel electrode, insulated except for the tip (tip: 0·5 mm; 0·3 mm in diameter); 48 h later lesions of the nucleus raphe medianus (MR) were produced in the same animals by applying a 2·5 mA anodal current for 17 s through a similar stainless-steel electrode. The stereotaxic coordinates (König & Klippel 1963) were: DR = A 0·4; L 0·0; H —0·6; MR = A 0·4; L 0·0; H —2·6. Controls were sham-operated by the same procedure, but not lesioned.

Ten days after lesion animals received [^{3}H]reserpine (NEN, 344 μCi mg^{-1}, >90% radiochemically pure) 1 mg kg^{-1} i.v. dissolved in citric acid, benzyl alcohol, polyethylene glycol and distilled water (Serpasil solvent) or (±)-fenfluramine HCl (5 mg kg^{-1} or 15 mg kg^{-1} i.p.).

The rats were decapitated at various intervals after drug injection and brains were quickly removed and dissected as described by Valzelli & Garattini (1968). Brain areas were wrapped in aluminium foil, frozen on dry ice and maintained at —25°C until assayed.

Blood (about 3 ml) was collected in a test tube containing 0·3 ml heparin dissolved in 0·9% NaCl (saline) (1 mg ml^{-1}), and centrifuged at 2000 *g* for 10 min. One ml plasma aliquots were frozen and stored at —25 °C until assayed. [^{3}H]Reserpine in tissue and plasma was assayed as described by Manara (1967), except for t.l.c. chromatography; each sample was spotted and developed on a glass rod, previously coated with silica gel (Manara et al 1972). The specificity of these [^{3}H]-reserpine measurements has been confirmed by a gas chromatographic-mass fragmentographic technique (Pantarotto et al 1976).

Samples were counted in a liquid scintillation spectrometer (Packard TriCarb 2425) with 45% counting efficiency.

Tissue and plasma concentrations of fenfluramine and norfenfluramine enantiomers were determined by gas-liquid chromatography (Caccia & Jori 1977).

No differences in concentrations of the enantiomers drug or of metabolite were found after either the 5 or 15 mg kg^{-1} dose of fenfluramine, even 8 h after injection, with most fenfluramine had disappeared from the brain. Conversely (Table 1) MR + DR lesion significantly reduced [^{3}H]reserpine concentrations in the hippocampus and telencephalon 24 h after 1 mg kg^{-1}, when the drug at low concentrations is persistently bound to nerve terminals.

Lesion of 5-HT-ergic neurons, which reportedly antagonizes the anorectic effect of 5 mg kg^{-1} (±)-fenfluramine (Samanin et al 1972), does not change the concentrations of the enatiomers of the drug or its metabolite in four different areas of the rat brain. This finding parallels our previous results with chlorimipramine, which prevents the effect of fenfluramine on brain 5-HT and food intake, without causing major changes in the concentrations of fenfluramine and its metabolite in brain (Ghezzi et al 1973).

In contrast, Belin et al (1976) reported that ten days after 5,6-HT treatment both the accumulation of [^{14}C]fenfluramine in the brain in vivo and its binding to synaptosomes in vitro were reduced. We found no such reduction in fenfluramine concentrations, even

* Correspondence.

Table 1. [^{3}H]Reserpine concentration (ng ml^{-1} or ng g^{-1}) after administration of 1 mg kg^{-1} i.v. to sham and MR + DR lesioned rats.

	1 h			24 h		
	A	B		A	B	
Sample	Sham	MR + DR	%B/A	Sham	MR + DR	% B/A
Plasma	45·6±3·5	44·6±0·5	98	n.d.	n.d.	
Striatum	130·3±10·6	115·2±0·9	88	94·8±6·2	91·2±3·7	96
Hypothalamus	47·3±1·5	58·6±1·6	124	27·2±2·1	23·6±4·2	87
Hippocampus	46·6±0·2	49·9±3·3	107	5·2±1·1	2·3±0·7*	44
Telencephalon	28·5±1·3	32·0±0·5	112	10·2±0·4	6·2±0·7**	61

Data are mean ± s.e. of 3–4 replications.
* $P < 0.05$ ** $P < 0.01$ Student's t-test.

after 15 mg kg^{-1} and 1 h after injection, a condition similar to that used by Belin et al (1976). However, this apparent discrepancy might arise from the fact that these authors measured total brain radioactivity and differences in metabolism between control and 5,6-HT lesioned animals could result in different ^{14}C concentrations, masking the true presence of fenfluramine. The real degeneration of 5-HT nerve terminals produced by MR + DR lesion is confirmed by our detection of a significant reduction of [^{3}H]reserpine concentrations in the hippocampus and telencephalon 24 h after injection. This indicates that the extensive degeneration of 5-HT-ergic neurons projecting to cortical regions of the brain affects [^{3}H]reserpine binding to nerve terminals located in this area.

The lack of effect in the striatum could be because the density of 5-HT terminals in this area is much less than that of dopamine-containing neurons (Fuxe 1965). Since [^{3}H]reserpine binds to catecholaminergic neurons in the brain (Mennini et al 1977), the prevalence of dopamine in the striatum could have masked the decrease in binding consequent to loss of 5-HT terminals in lesioned rats. This argument is not valid for the hypothalamus, where there are no marked differences in the density of 5-HT and noradrenaline nerve terminals (Fuxe et al 1975). Since the reduction of [^{3}H]5-HT uptake found in the hypothalamus was similar to that in the hippocampus of MR + DR lesioned rats (about 70%, unpublished results), the failure of MR + DR lesion to change hypothalamic [^{3}H]reserpine binding does not seem to be explained by a less marked degeneration of 5-HT nerve terminals in this area; it might however be linked to reserpine binding to different neuronal populations.

The reserpine data show that it is possible to detect changes of drug binding in some brain areas of rats with extensive degeneration of central 5-HT neurons. The lack of effect of MR + DR lesion on fenfluramine and norfenfluramine concentrations indicates that fenfluramine interacts with central 5-HT neurons through a mechanism different from that of reserpine, or else that binding is so short-lasting that appropriate experimental conditions to reveal it were not achieved.

The possibility of fenfluramine localizing in catecholaminergic neurons, similar to reserpine (Mennini et al 1977), can also be excluded since an intraventricular injection of 6-hydroxydopamine does not change concentrations of the drug or metabolite enantiomers at different times after injection of 5 or 15 mg kg^{-1} (±)-fenfluramine (unpublished results).

October 26, 1979

REFERENCES

Alphin, R. S., Ward, J. W. (1969) Toxicol. Appl. Pharmacol. 14:182–191

Belin, M.-F., Kouyoumdjian, J.-C., Bardakdhan, J., Duhault, J., Gonnard, P. (1976) Neuropharmacology 15:613–617

Baumgarten, H. G., Björklund, A., Lachenmayer, L., Nobin, A., Stenevi, U. (1971) Acta Physiol. Scand. Suppl. 373, 1–15

Caccia, S., Jori, A. (1977) J. Chromatogr. 144:127–131

Fuxe, K. (1965) Acta Physiol. Scand. 64: Suppl. 247, 37–85

Fuxe, K., Farnebo, L. O., Hamberger, B., Ogren, S. O. (1975) Postgrad. Med. J. 51:Suppl. 1, 35–45

Garattini, S., Borroni, E., Mennini, T., Samanin, R. (1978) in: Garattini, S., Samanin, R. (eds) Central Mechanisms of Anorectic Drugs, Raven Press, New York, pp 127–143

Garattini, S., Caccia, S., Mennini, T., Samanin, R., Consolo, S., Ladinsky, H. (1979) Curr. Med. Res. Opin. 6:Suppl. 1, 15–27

Ghezzi, D., Samanin, R., Bernasconi, S., Tognoni, G., Gerna, M., Garattini, S. (1973) Eur. J. Pharmacol. 24:205–210

Kannengiesser, M.-H., Hunt, P. F., Raynaud, J.-P. (1976) Ibid. 35:35–43

König, J. F. R., Klippel, R. A. (1963) The Rat Brain: A Stereotaxic Atlas of the Forebrain and Lower Parts of the Brain Stem. Williams and Wilkins, Baltimore

Le Douarec, J. C., Schmitt, H. (1964) Therapie 19:831–841

Manara, L. (1967) Eur. J. Pharmacol. 2:136–138

Manara, L., Bareggi, S. R., Cerletti, C., Luzzani, F., Mennini, T. (1974) in: Morselli P. L., Garattini, S., Cohen, S. N. (eds) Drug Interactions, Raven Press, New York, pp 135–150

Manara, L., Mennini, T., Carminati, P. (1972) Eur. J. Pharmacol. 17:183–185

Mennini, T., Bernasconi, S., Manara, L., Samanin, R., Serra, G. (1977) Pharmacol. Res. Commun. 9: 857–862

Pantarotto, C., Belvedere, G., Frigerio, A., Mennini, T., Manara, L. (1976) Eur. J. Drug. Metab. Pharmacokinet. 1:25–34

Samanin, R., Ghezzi, D., Valzelli, L., Garattini, S. (1972) Eur. J. Pharmacol. 19:318–322

Valzelli, L., Garattini, S. (1968) J. Neurochem. 15:259–261

Effect of systemic and intrastriatal injections of haloperidol on striatal dopamine and DOPAC concentrations in rats pretreated by section of nigrostriatal fibres

S. M. WUERTHELE*, K. E. MOORE†, *Department of Pharmacology and Toxicology Michigan State University, East Lansing, Michigan 48824, U.S.A.*

Systemic administration of dopamine receptor antagonists increases, while dopamine agonists decrease the activity of nigrostriatal dopaminergic neurons (Carlsson & Lindqvist 1963; Bunney et al 1973a,b). It has generally been assumed that the changes in nigrostriatal activity induced by systemic administration of dopamine agonists and antagonists result primarily from activation of dopamine receptors located either on dopaminergic cell bodies or dendrites in substantia nigra (autoreceptors), on cell bodies or dendrites postsynaptic to dopaminergic terminals in the striatum (postsynaptic receptors) or on striatal dopaminergic terminals (presynaptic receptors).

According to the presynaptic receptor hypothesis (Christiansen & Squires 1974; Nowycky & Roth 1978; Farnebo & Hamberger 1970; Reimann et al 1979), activation of dopamine receptors located on dopamine nerve terminals or axons in the striatum inhibits synthesis and release of dopamine. On the other hand, stimulation of postsynaptic dopamine receptors has been postulated (Carlsson & Lindqvist 1963) to control nigrostriatal activity by activation of an inhibitory striatonigral feedback loop. That such a loop mediates changes in nigrostriatal activity produced by systemic administration of dopamine agonists and antagonists, has recently been questioned. After kainic acid-induced destruction of the striatal cell bodies forming this loop, nigrostriatal neurons still respond to dopamine agonists with decreased, and to dopamine antagonists with increased activity, as estimated either by changes in striatal dihydroxyphenylacetic acid (DOPAC) concentrations, or by the α-methyl-*p*-tyrosine-induced decline of striatal dopamine concentrations (Di Chiara et al 1977; Wuerthele & Moore 1979). Experiments with section or electrolytic lesions of the nigrostriatal fibres (Bedard & Larochelle 1973; Garcia-Munoz et al 1977) also suggest that a feedback loop is not essential for changes in nigrostriatal activity induced by dopamine agonists and antagonists. The following report supports this conclusion and presents evidence that the increases in nigrostriatal activity produced by the systemic administration of dopamine antagonists are only partially due to the blockade of presynaptic striatal receptors.

Male Sprague-Dawley rats (200–300 g, Spartan Research Animals, Haslett, Michigan) were anaesthetized with Equithesin (3 ml kg^{-1}). Sections of the striatonigral path was effected by lowering a 2·2 mm wide stainless steel blade to the base of the skull 2·2 mm anterior to the intra-aural line, with the medial edge of the blade 1·8 mm lateral to the midline (König & Klippel 1963). Hemitranssections were made by slowly moving this knife laterally from the midline 4·8 mm. In some animals, 23 gauge cannula guides were permanently implanted into the striatum (2·0 mm anterior to Bregma, ± 3·0 mm lateral from the midline and 4·3 mm ventral from the dura (Pellegrino & Cushman 1967). Injections were made through these cannula guides by inserting 30 gauge injector cannulae into the implanted guides, so that the tip extended 1 mm below the tip of the guide cannula. Drugs were injected over 2 min from a 5 μl syringe mounted on an infusion pump and connected to the cannulae by short pieces of polyethylene tubing. Animals were decapitated, and brains rapidly removed and dissected on a thermoelectric cold plate. Pieces of tissue containing the striatum and substantia nigra were frozen on dry ice, sliced and further dissected with the aid of a stereoscope. Striatal and nigral concentrations of dopamine and DOPAC were measured by a radioenzymatic method (Umezu & Moore 1979). Nigral glutamic acid decarboxylase (GAD) activity was measured by a modification of the method of Kanazawa et al (1976). Significance of the differences between means were tested using two-way analysis of variance (Sokol & Rohlf 1969). Regression analysis was used to test the correlation between nigral GAD activities and increases in striatal DOPAC concentrations (Table 3). Injectable haloperidol (Haldol) and its vehicle were supplied by Dr J. Plostnieks, McNeil Laboratories, Ft. Washington, Pennsylvania. Sulpiride was obtained from Ravizza Research Laboratories, Milan, Italy.

Because the nigrostriatal and striatonigral fibres lie in close proximity, it is not possible to make completely selective lesions of the descending striatonigral fibres. Therefore, only rats in which sectioning produced less

* Present address: Department of Pharmacology, C236 University of Colorado Health Sciences Center, 4200 E. Ninth Ave. Denver, CO 80262, U.S.A.

† Correspondence.