J. Pharm. Pharmacol. 1981, 33: 538–540
Communicated March 23, 1981

0022–3573/81/080538–03 $02.50/0

Different action of 5-hydroxytryptamine (5-HT) uptake inhibitors on fenfluramine- but not *p*-chloramphetamine-induced hyperthermia in rats

LESZEK PAWŁOWSKI, *Institute of Pharmacology, Polish Academy of Sciences, 12 Smetna Street, 31–343 Kraków, Poland*

Fenfluramine and *p*-chloroamphetamine (PCA), two 5-HT-mimetics with an indirect mechanism of action, produce a pronounced, centrally mediated hyperthermia in rats kept at 28 °C (Frey 1975; Sulpizio et al 1978; Pawłowski et al 1980a,b). This hyperthermia is counteracted by drugs blocking the 5-HT receptor or 5-HT synthesis as well as by clomipramine, a potent and selective inhibitor of the 5-HT uptake (Frey 1975; Sulpizio et al 1978; Pawłowski et al 1980a). Fluoxetine, another selective 5-HT uptake inhibitor, has been reported to prevent both the fenfluramine- and PCA-induced hyperthermia in rabbits (Quock & Weick 1979); thus it is assumed that the drugs blocking the uptake of 5-HT should simultaneously counteract the fenfluramine- and PCA-induced hyperthermia, presumably by blocking the entrance of the 5-HT-releasing agents into 5-HT neurons.

Recently some new inhibitors of the 5-HT uptake have been developed. One of them, zimelidine—a drug more potent and selective than clomipramine in several appropriate tests in vivo (Ross & Renyi 1975; Ross et al 1976a,b; Buus Lassen 1978), was reported not to prevent the fenfluramine-induced hyperthermia but to counteract the hyperthermia induced by PCA (Pawłowski et al 1980a,b). This challenged an opinion that the mechanism of action of PCA and fenfluramine on the 5-HT neuron membrane is identical and, in particular, indicated that the test based on counteracting the hyperthermic effect of fenfluramine may not always result in detecting an inhibitory activity of the 5-HT uptake.

The action of a few compounds described as potent and selective inhibitors of the 5-HT uptake on the hyperthermic effect induced by PCA and fenfluramine at a high ambient temperature in the rat has been examined. The compounds used were: citalopram (Lu 10-171) (Christensen et al 1977; Hyttel 1977), femoxetine (FG 4963) (Buus Lassen et al 1975a,b), Org 6582 [(±)-8-chloro-11-anti-amino-benzo-(b)-bicyclo-(3,3,1)-nona-3,6a-(10a)-diene hydrochloride] (Goodlet et al 1976; Sugrue et al 1976; Mireylees et al 1978) and Ro 11-2465 [5-(3-(dimethylamino) propyl)-10,11-dihydro-5-H-dibenz(b,f)azepine-3-carbonitrile] (Haefely et al 1978). Except for citalopram, the drugs prevented the pharmacological and/or biochemical effects of PCA (Buus Lassen et al 1975a,b; Goodlet et al 1976; Haefely et al 1978). Citalopram antagonized the effects of another 5-HT-releasing agent, H 75/12 (α-ethyl-4-methyl-*m*-tyramine), which also produced hyperthermia in the rat and depleted the brain of 5-HT (Christensen et al 1977). None of these drugs have been tested with respect to the fenfluramine-induced behavioural or biochemical effects. Clomipramine, a classical inhibitor of the 5-HT uptake, served as a reference compound.

Male Wistar rats (160–220 g), before the experiment were adapted for 1 h to the experimental conditions (temp. 28 ± 1 °C, humidity 40%). The oesophageal body temperature was measured with an Ellab T-3 thermometer. Drugs were dissolved in 0·9% NaCl (saline) and injected i.p. in a volume of 4 ml kg^{-1}. The inhibitors of the 5-HT uptake (or saline) were administered 1 h before PCA or fenfluramine. Each group consisted of 6 animals. The statistical evaluation was using Student's *t*-test.

The 5-HT uptake inhibitors did not change the basal body temperature of rats kept at a high ambient temperature; the only exception was Ro 11-2465 which elevated the temperature in a dose-dependent manner (the maximal increase observed after a dose of 40 mg kg^{-1} was: +1·7 °C) (data not shown). All the compounds, including Ro 11-2465, significantly inhibited the hyperthermia induced by PCA (Table 1). However, the drugs acted differently on the fenfluramine-induced hyperthermia: femoxetine was the most potent in inhibiting the hyperthermia, citalopram was as active as clomipramine, whereas Org 6582 was ineffective (Table 2). Similarly, no significant effect was produced by doses of Ro 11-2465 effective in abolishing the hyperthermic effect of PCA; only a dose of 40 mg kg^{-1} evoked a significant inhibitory effect (Table 2).

The present results, as well as those of the previous studies (Pawłowski et al 1980a,b), indicate that the so-called specific 5-HT uptake inhibitors do not constitute a homogeneous group. All these compounds are able to counteract the accumulation of 5-HT from the medium in 5-HT-ergic nerve terminals and apparently exert the same effect on the uptake of PCA, thus preventing the releasing action of the latter drug on 5-HT contained in nerve endings. However, only some of these drugs counteract the action of fenfluramine.

At present it is not clear whether the ineffectiveness of such compounds as zimelidine (Pawłowski et al 1980a,b) and Org 6582 in counteracting the effect of fenfluramine is due to their lack of action in preventing the uptake of fenfluramine, or to some unknown factors. Nevertheless, the ineffectiveness of zimelidine and Org 6582 in the fenfluramine-induced hyperthermia is of a practical

Table 1. Effect of selective inhibitors of the 5-HT uptake (given 1 h before) upon *p*-chloroamphetamine (PCA)-induced hyperthermia in rats kept at the ambient temperature of 28 ± 1 °C.

Treatment mg kg^{-1} i.p.		Change from baseline oesophageal temperature at x min after challenge (°C ± s.e.m.)				
		x = 30	60	90	120	150
Saline		0·2 ± 0·12	0·4 ± 0·12	0·3 ± 0·13	0·1 ± 0·08	0·1 ± 0·15
PCA	(6·5)	2·2 ± 0·23	2·3 ± 0·10	2·0 ± 0·18	2·2 ± 0·11	2·4 ± 0·15
Clomipramine	(20)	0·7 ± 0·23***	1·0 ± 0·25***	1·4 ± 0·23	1·6 ± 0·30	1·4 ± 0·26*
+ PCA	(6·5)					
Citalopram	(20)	0·6 ± 0·10***	1·4 ± 0·22***	1·7 ± 0·29	2·1 ± 0·42	2·2 ± 0·39
+ PCA	(6·5)					
Femoxetine	(20)	0·4 ± 0·12***	1·0 ± 0·12***	1·0 ± 0·16***	1·2 ± 0·17***	1·1 ± 0·13***
+ PCA	(6·5)					
Org 6582	(20)	0·5 ± 0·06***	1·2 ± 0·22***	1·2 ± 0·20*	1·4 ± 0·16**	1·4 ± 0·15**
+ PCA	(6·5)					
Ro 11-2465	(10)	1·1 ± 0·20**	1·3 ± 0·17***	1·3 ± 0·15*	1·4 ± 0·16**	1·3 ± 0·14***
+ PCA	(6·5)					
Ro 11-2465	(20)	1·1 ± 0·10**	1·5 ± 0·10***	1·7 ± 0·17	1·8 ± 0·12*	1·7 ± 0·18*
+ PCA	(6·5)					

Each experimental group consisted of 6 rats.
* $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$ (Difference from group receiving PCA alone; Student's *t*-test).

Table 2. Effect of selective inhibitors of 5-HT uptake (given 1 h before) upon fenfluramine-induced hyperthermia in rats kept at the ambient temperature of 28 ± 1 °C.

Treatment mg kg^{-1} i.p.		Change from baseline oesophageal temperature at x min after challenge (°C ± s.e.m.)				
		x = 30	60	90	102	150
Saline		0·2 ± 0·10	0·3 ± 0·09	0·2 ± 0·09	0·1 ± 0·07	0·2 ± 0·12
Fenfluramine	(20)	1·3 ± 0·31	1·8 ± 0·31	1·8 ± 0·15	2·0 ± 0·19	2·0 ± 0·20
Clomipramine	(5)	1·7 ± 0·22	2·0 ± 0·23	1·9 ± 0·18	1·9 ± 0·16	1·7 ± 0·26
+ fenfluramine	(20)					
Clomipramine	(10)	1·2 ± 0·19	1·5 ± 0·14	1·7 ± 0·13	1·9 ± 0·16	1·9 ± 0·12
+ fenfluramine	(20)					
Clomipramine	(20)	0·3 ± 0·08*	0·7 ± 0·10**	1·0 ± 0·10**	1·2 ± 0·11**	1·4 ± 0·13*
+ fenfluramine	(20)					
Citalopram	(5)	1·1 ± 0·15	1·6 ± 0·17	1·6 ± 0·23	1·7 ± 0·23	1·8 ± 0·38
+ fenfluramine	(20)					
Citalopram	(10)	0·8 ± 0·25	1·2 ± 0·24	1·5 ± 0·20	1·7 ± 0·25	1·9 ± 0·32
+ fenfluramine	(20)					
Citalopram	(20)	0·2 ± 0·25*	0·3 ± 0·25*	0·6 ± 0·26*	0·6 ± 0·30*	0·9 ± 0·38*
+ fenfluramine	(20)					
Femoxetine	(5)	0·6 ± 0·15	1·5 ± 0·22	1·7 ± 0·24	1·7 ± 0·27	1·6 ± 0·24
+ fenfluramine	(20)					
Femoxetine	(10)	0·4 ± 0·11*	0·8 ± 0·18*	1·0 ± 0·17**	1·2 ± 0·19**	1·3 ± 0·17*
+ fenfluramine	(20)					
Femoxetine	(20)	−0·2 ± 0·13**	0·1 ± 0·15***	0·4 ± 0·19***	0·7 ± 0·24**	1·0 ± 0·23**
+ fenfluramine	(20)					
Org 6582	(10)	1·3 ± 0·18	1·7 ± 0·16	1·8 ± 0·15	2·0 ± 0·12	2·1 ± 0·15
+ fenfluramine	(20)					
Org 6582	(20)	1·0 ± 0·17	1·6 ± 0·13	1·7 ± 0·12	1·8 ± 0·12	1·8 ± 0·12
+ fenfluramine	(20)					
Org 6582	(40)	1·8 ± 0·27	1·9 ± 0·14	1·9 ± 0·14	2·1 ± 0·22	2·1 ± 0·18
+ fenfluramine	(20)					
Ro 11-2465	(10)	1·1 ± 0·10	1·4 ± 0·17	1·6 ± 0·13	1·7 ± 0·17	1·9 ± 0·23
+ fenfluramine	(20)					
Ro 11-2465	(20)	0·8 ± 0·22	1·3 ± 0·22	1·3 ± 0·15	1·6 ± 0·22	1·8 ± 0·20
+ fenfluramine	(20)					
Ro 11-2465	(40)	0·6 ± 0·16	0·7 ± 0·15*	0·9 ± 0·12**	1·1 ± 0·20*	1·3 ± 0·23*
+ fenfluramine	(20)					

Each experimental group consisted of 6 rats.
* $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$ (Difference from group receiving fenfluramine alone; Student's *t*-test).

interest. The results of Sulpizio et al (1978) suggest that the fenfluramine-induced hyperthermia in the rat may be a good test for the 5-HT uptake inhibition by drugs. The present results indicate that the test is not as universal as originally thought, since it yields false negative results for some compounds, such as zimelidine and Org 6582. On the other hand, the hyperthermia test employing PCA seems in more cases to give results parallel to those obtained in in vitro tests for 5-HT uptake inhibition.

I would like to thank the following companies for the generous gift of substances: A/S Ferrosan for femoxetine, Ciba Geigy for clomipramine, H. Lundbeck & Co. for citalopram, Hoffman La Roche for Ro 11-2465, Les Laboratoires Servier for fenfluramine and Organon for Org 6582.

REFERENCES

Buus Lassen, J., Petersen, E., Kjellberg, B., Olsson, S. O. (1975a) Eur. J. Pharmacol. 32: 108–115

Buus Lassen, J., Squires, R. F., Christensen, J. A., Molander, L. (1975b) Psychopharmacologia (Berlin) 42: 21–26

Buus Lassen, J. (1978) Eur. J. Pharmacol. 47: 351–358

Christensen, A. V., Fjalland, B., Pedersen, V., Danneskiold-Samsøe, P., Svedsen, O. (1977) Eur. J. Pharmacol. 41: 153–162

Frey, H. H. (1975) Pharmacology 13: 163–176

Goodlet, I., Mireylees, S. E., Sugrue, M. F. (1976) Br. J. Pharmacol. 56: 367P–368P

Haefely, W., Schaffner, R., Burkard, W. P., Da Prada, M., Keller, H. H., Polc, P., Richards, J. G. (1978) in: Abstracts of the 11th CINP Congress, Vienna, 9–14 July 1978, p. 95.

Hyttel, J. (1977) Psychopharmacology 51: 225–233

Mireylees, S. E., Goodlet, I., Sugrue, M. F. (1978) Biochem. Pharmacol. 27: 1023–1027

Pawłowski, L., Kwiatek, H., Ruczyńska, J. (1980a) in: Abstracts of the 12th CINP Congress, Göteberg, 22–26 June 1980, Progr. Neuro-Psychopharmacol., suppl., Pergamon Press, Oxford 1980, p. 277, abstr. 516

Pawłowski, L., Ruczyńska, J., Maj, J. (1980b) Neurosci. Lett., 16: 203–207

Quock, S. B., Weick, B. G. (1979) J. Pharm. Pharmacol. 31: 27–32

Ross, S. B. Renyi, A. L. (1975) Acta Pharmacol. (Kbh) 36: 395–408

Ross, S. B., Jansa, S., Wetterberg, L., Fyrö, B., Hellner, B. (1976a) Life Sci. 19: 205–210

Ross, S. B., Ögren, S.-O., Renyi, A. L. (1976b) Acta Pharmacol. (Kbh) 39: 152–166

Sugrue, M. F., Goodlet, I., Mireylees, S. E. (1976) Eur. J. Pharmacol. 40: 121–130

Sulpizio, A., Fowler, P. J., Macko, E. (1978) Life Sci. 22: 1439–1446

J. Pharm. Pharmacol. 1981, 33: 540–543
Communicated January 26, 1981

0022–3573/81/080540–04 $02.50/0

The reduction of tuberculin-induced pleurisy in the guinea-pig by a gold salt, chloroquine and D-penicillamine

J. P. TARAYRE*, M. BRU, V. CAILLOL, M. VIALADE, H. LAURESSERGUE, *Department of Pharmacology, P. Fabre Research Centre, 17, avenue J. Moulin, 81100 Castres, France*

It is well established that the phenomena of delayed hypersensitivity play a major role at the synovial membranes of the affected joints in rheumatoid arthritis. In the search for models in which this type of hypersensitivity is involved and reduced by antirheumatic agents, we have studied the effects of chloroquine, D-penicillamine and a gold salt, sodium aurothiopropanol sulphonate, on a classic model of delayed hypersensitivity, purified-protein-derivative (PPD) pleurisy in the guinea-pig (Allen & Apicella 1968).

Material and methods

Sodium aurothiopropanol sulphonate (Sarbach); chloroquine diphosphate (Rhône Poulenc); D-penicillamine (Fluka).

Dukin-Hartley guinea pigs of either sex, 300–400 g, and male Swiss mice, 23–25 g, were used.

Tuberculin-induced pleurisy in guinea-pigs. 80 mg of killed *Mycobacterium tuberculosis* (Difco) was ground in a mortar and was then suspended in a mixture of 10 ml of incomplete Freund's adjuvant (Difco) and 10 ml of a 0·2 M phosphate buffer solution, pH: 7. 0·5 ml of this suspension was injected into the thigh muscles of the guinea-pigs.

Five to 7 weeks after the sensitization, batches of animals were made homogeneous in weight and sex (50% males and 50% females). PPD (Institut Pasteur) 10 μg 0·1 ml was injected into the pleural cavity of the animals lightly anaesthetized with ether. 48 h later, the animals were killed and the volume of the pleural exudate was measured. The pleural cavity was rinsed with 2 ml of medium 199 (Institut Pasteur) to recover the cells remaining on the walls, and the leucocytes of the exudate were counted with a Coulter Counter (Coultronics, model ZF). The samples were spread on slides and stained with Hemacolor (Merck), and the numbers of mononuclear cells, neutrophils, and eosinophils determined.

The antirheumatic agents were dissolved in 0·9% NaCl, and D-penicillamine and chloroquine diphosphate were injected subcutaneously, the gold salt intramuscularly. Controls received the solvent alone. The agents were administered 24 and 2 h before and 2 and 24 h after the challenge.

Action of antirheumatic agents on circulating blood cells. Groups of animals containing 50% males and

* Correspondence.