J. Pharm. Pharmac., 1974, **26**, 814

COMMUNICATIONS

Influence of some tricyclic antidepressive drugs on the uptake of 5-hydroxytryptamine by rat blood platelets

Several studies have shown that tricyclic antidepressive drugs inhibit the uptake of biogenic amines in the central nervous system; some of these drugs show a certain selectivity in inhibiting either the catecholamine or the 5-hydroxytryptamine (5-HT) uptake (Carlsson, Fuxe & others, 1966; Carlsson, Corrodi & others, 1969; Ross & Renyi, 1967, 1969).

The influence of tricyclic antidepressive drugs on catecholamine uptake has been studied directly on nerve endings; much of this work has been with rats (Garattini, Bonaccorsi & others, 1972; Hamberger & Tuck, 1973); in contrast, 5-HT uptake has been mainly investigated in blood platelets which are considered as a useful pharmacological model for serotoninergic neurons; human and rabbit platelets have generally been used for this purpose (Paasonen, 1968; Pletscher, 1968; Todrick & Tait, 1969; Tuomisto, 1974). We have evaluated the activity of tricyclic antidepressive drugs on 5-HT uptake in rat platelets.

Blood was obtained from ether anaesthetized Sprague Dawley rats by intracardiac puncture using a 10 ml disposable syringe containing 1 ml 3·13% trisodium citrate. Platelet-rich plasma (PRP) was obtained by centrifugation of blood at 400 *g* for 15 min; platelets were counted by phase microscopy and adjusted to about 600 000 μl^{-1} by appropriate dilutions with autologous platelet-poor plasma obtained by centrifuging blood at 3000 *g* for 15 min. PRP (1·9 ml) was preincubated for 15 min at 37° with 0·1 ml buffer or drug solutions; afterwards, 0·2 mg ^{14}C-5-HT was added and PRP was incubated for an additional 15 min; the samples were gently shaken during the incubation period. Samples were then cooled in an ice bath and subsequently centrifuged in a cold room at 3000 *g* for 20 min, the supernatant plasma being collected. Radioactivity was counted for 1 min in a liquid scintillation counter (Beckman LS-250) after addition of uncentrifuged PRP or supernatant 0·2 ml to 15 ml of a dioxane-naphthalene scintillation mixture. As rat platelets do not contain monoamine oxidase (Paasonen, 1965), it was assumed that radioactivity was a measure of ^{14}C-5-HT uptake. Uptake of ^{14}C-5-HT was calculated by the following formula:

$$\text{Uptake } \% = \frac{(\text{Counts min}^{-1}\text{ PRP} - \text{counts min}^{-1}\text{ supernatant})}{\text{Counts min}^{-1}\text{ PRP}} \times 100$$

5-Hydroxytryptamine-3'[^{14}C]creatinine sulphate (55 mCi mmol^{-1}) was obtained from the Radiochemical Centre, Amersham, and dissolved in 70% ethanol; doxepine HCl *trans* and *cis*-forms and desmethyldoxepine HCl were from Pennwalt Corporation, Rochester, U.S.A., imipramine HCl, 3-chlorimipramine, desipramine, *N*-desmethyl-3-chlorimipramine HCl were from Ciba-Geigy, Basel, Switzerland; amitriptyline HCl and nortriptyline (Merck, Sharp & Dohme, Rahway, N.J., U.S.A.) were dissolved in physiological saline to give a final concentration of $2{\cdot}5 \times 10^{-5}$M. This is the concentration of chlorimipramine (chosen as reference drug) which in preliminary experiments gave almost complete inhibition of ^{14}C-5-HT uptake by rat blood platelets.

The results obtained are reported in Table 1. Chlorimipramine was the most potent inhibitor, followed by imipramine, desmethylchlorimipramine and amitriptyline; all these drugs inhibited 5-HT uptake by more than 50%; in contrast, nortripty-

Table 1. *Influence of tricyclic antidepressive drugs ($2{\cdot}5 \times 10^{-5}$M) on the uptake of ^{14}C-5-HT by rat blood platelets.* Mean ± s.d. of at least 4 experiments.

Drugs	% Inhibition of ^{14}C-5-HT uptake
Chlorimipramine	89·0 ± 3·6
Imipramine	73·0 ± 5·2
Desmethylchlorimipramine	65·0 ± 6·8
Amitriptyline	61·7 ± 5·2
Nortriptyline	37·2 ± 8·6
trans-Doxepine	33·2 ± 1·1
Doxepine	28·0 ± 3·0
Desipramine	23·0 ± 8·1
Desmethyldoxepine	12·9 ± 2·3
cis-Doxepine	3·5 ± 1·7

line, doxepine, desipramine and desmethyldoxepine inhibited the uptake of ^{14}C-5-HT by less than 50%. For each drug, the tertiary amine appeared to be more active than the corresponding metabolite (secondary amine). As the doxepine used was a mixture of about 15% *cis*- and 85% *trans*-doxepine, the effect of both components was separately investigated. The *trans*-compound proved to be at least as active as the mixture and the *cis*-compound was almost devoid of inhibitory activity. Our results are in agreement with those of similar experiments with human and rabbit blood platelets; however, the relative potency of some drugs compared to chlorimipramine, is not identical; e.g. nortriptyline was more active than desipramine in rat and rabbit but not in human platelets (Todrick & Tait, 1969; Tuomisto,1974). The present results indicate that rat platelets would be useful as a model for evaluating the rank order of the inhibitory potency of tricyclic antidepressive drugs on ^{14}C-5-HT uptake mechanisms.

This work was partially supported by C.N.R.—Contracts No. 73.00218.31 and No. 73.00400.04. We thank Pennwalt Corporation, Ciba Geigy and Merck Sharp & Dohme who provided the drugs.

Istituto di Richerche
Farmacologiche "Mario Negri,"
Via Eritrea, 62,
20157 Milano, Italy

W. Buczko*
G. de Gaetano**
S. Garattini

May 7, 1974

* Visiting Scientist from the Department of Pharmacology, Medical School, Białystok, Poland.
** To whom reprint requests should be addressed.

REFERENCES

Carlsson, A., Corrodi, H., Fuxe, H. & Hökfelt, T. (1969). *Eur. J. Pharmac.*, **5**, 367–373.
Carlsson, A., Fuxe, K., Hamberger, B. & Lindqvist, M. (1966). *Acta physiol. scand.*, **67**, 481–497.
Garattini, S., Bonaccorsi, A., Jori, A. & Samanin, R. (1972). *Revue Neurol.*, **127**, 265–292.
Hamberger, B. & Tuck, J. R. (1973). *Eur. J. clin. Pharmac.*, **5**, 229–235.
Paasonen, M. K. (1965). *J. Pharm. Pharmac.*, **17**, 681–697.
Paasonen, M. K. (1968). *Annls Med. exp. Biol. Fenn.*, **46**, 416–422.
Pletscher, A. (1968). *Br. J. Pharmac.*, **32**, 1–16.
Ross, S. B. & Renyi, A. L. (1967). *Eur. J. Pharmac.*, **2**, 181–186.
Ross, S. B. & Renyi, A. L. (1969). *Ibid.*, **7**, 270–277.
Todrick, A. & Tait, A. C. (1969). *J. Pharm. Pharmac.*, **21**, 751–762.
Tuomisto, J. (1974). *Ibid.*, **26**, 92–100.