Improved method for evaluating the inhibition of [^{14}C]5-hydroxytryptamine uptake by rat platelets

M. WIELOSZ*, G. DE GAETANO†, S. GARATTINI, *Laboratory for Haemostasis and Thrombosis Research, Istituto di Ricerche Farmacologiche 'Mario Negri', Via Eritrea, 62, 20157 Milan, Italy*

We have previously shown that rat platelets may be a useful model for evaluating the inhibitory potency of tricyclic antidepressant and anorectic drugs on ^{14}C-5-HT uptake mechanisms (Buczko, de Gaetano & Garattini, 1974, 1975; Garattini, de Gaetano & others, 1976). However, the concentrations of some of these drugs required to inhibit ^{14}C-5-HT uptake by rat platelets by 50% were relatively much higher than those required to obtain a similar inhibitory effect on rat brain slices (Ross & Renyi, 1975) or brain stem synaptosomes (Tuomisto, 1974).

Recently, Tuomisto (1974) demonstrated that, by using low substrate concentrations and short incubation times, 5-HT uptake in rabbit platelets and its pharmacological inhibition could be more comparable to that occurring in rat synaptosomes.

Our aim was to evaluate whether by modifying the technique previously used to study ^{14}C-5-HT uptake by rat platelets, the relative potency of inhibitory drugs would appear to be greater, as suggested by Tuomisto's work. We therefore determined the experimental conditions in which the measured rate of ^{14}C-5-HT uptake would represent more accurately the initial velocity of the reaction (for details see Wielosz, Salmona & others, 1976).

Compared to previously used techniques, the main difference appeared to be the incubation period of platelets with ^{14}C-5-HT. Indeed, we found that its uptake was apparently linear only between 10 and 60 s and reached a plateau within 60–120 s. This observation clearly indicated that the incubation periods used previously (15 min, Buczko & others, 1974, 1975, or 2 min, Garattini & others, 1976) were too long, although shorter than those generally used by others (Stacey, 1961; Yates, Todrick & Tait, 1964; Fuks, Lanman & Schanker, 1964). The incubation period used in the present study was therefore limited to 30 s. The substrate concentration (0·5 μM) used in our previous studies appeared to be adequate, being within the range of the measured Km value (0·48–0·62 μM).

In all our studies, platelets were preincubated at 37° for 10 min, a period long enough to allow optimal temperature equilibration (Praga & Pogliani, 1973). Unlike Tuomisto (1974), we did not dilute platelet-rich plasma with any buffer since we preferred to make our study in a more physiological condition. (Tuomisto himself, 1974 found considerable variation in uptake by using either a bicarbonate or a phosphate buffer.)

Platelet number was kept at about 600 000 μl^{-1}. Although a lower platelet concentration would seem more suitable for kinetic studies (Tuomisto, 1974), we decided to keep it at the concentration previously used, the better to compare the influence of variable time in our system.

Drugs used were: chlorimipramine HCl, imipramine HCl (Ciba-Geigy, Basel, Switzerland); Lilly 110140 (3-(*p*-trifluoromethylphenoxy)-*N*-methyl-3-phenylpropylamine, Eli -Lilly, Indianapolis, U.S.A.), (+)fenfluramine (Servier, Paris, France), trazodone and *m*-chlorophenylpiperazine (Angelini, Roma, Italy) and (+)-amphetamine (Recordati, Milano, Italy).

Table 1 shows the IC50 values obtained for these drugs in the experimental conditions defined above, or used previously. For the sake of comparison, IC50 values available in the literature for some of these drugs when studied on 5-HT uptake by rat synaptosomes are also included.

As can be seen, the level of potency of the whole group was more than 10 times higher in the present study compared to the results obtained with 15 min incubation. The values observed by stopping the reaction after 2 min were only slightly higher than those reported here. The ranking of inhibitory potency, however, was the same in the three different experimental conditions used. The relative potency of chlorimipramine, imipramine, Lilly 110140 and (+)-fenfluramine was virtually the same whether studied on platelets or on synaptosomes. Indeed, in both systems, chlorimipramine appeared to be about 10 times more

Table 1. *Comparison of the inhibitory effect of drugs on ^{14}C-5-HT uptake by rat platelets and rat brain synaptosomes.*

Drugs	Platelets IC50 (μM) 30 s	2 min	15 min	Synaptosomes (IC50 μM)
Chlorimipramine	0·16	0·36	2·3	0·02[a]–0·09[b]
Lilly 110140	0·28	0·24	3·2	0·06[b]
Imipramine	1·25	—	—	0·1[c]–0·5[b]
(+)Fenfluramine	1·30	2·90	25·0	0·5[c]
m-Chlorophenylpiperazine	3·00	7·00	36·0	—
Trazodane	8·50	12·50	64·0	—
(+)-Amphetamine	50·0	—	>100·0	—

Concentrations producing 50% inhibition (IC50) of ^{14}C-5-HT uptake were determined from log dose-response curves based on three to five experiments using three different concentrations of drugs. For sake of comparison, last column gives IC50 values of some drugs on 5-HT uptake by rat brain synaptosomes.
(a) Tuomisto, 1974.
(b) Wong, Bymaster & others, 1975.
(c) Fuxe, Farnebo & others, 1975.

* Visiting Scientist from the Department of Pharmacology, Institute of Clinical Pathology, Medical School, Lublin, Poland.
† Correspondence.

effective than imipramine or fenfluramine and as active as Lilly 110140. These data are in close agreement with those recently presented by Tuomisto (1974) and add further support to the usefulness of platelets as a model for the study of serotoninergic nerve endings.

Possibly, the methodology for measuring 5-HT uptake by platelets and its pharmacological inhibition can be further improved (e.g. by shortening the incubation period even more and/or reducing the platelet number and/or stopping the reaction by means more effective than lowering the temperature).

This work was partially supported by Grant CNR 73. 00218. 31. March 9, 1976

REFERENCES

BUCZKO, W., DE GAETANO, G. & GARATTINI, S. (1974). *J. Pharm. Pharmac.*, **26**, 814–815.

BUCZKO, W., DE GAETANO, G. & GARATTINI, S. (1975). *Ibid.*, **27**, 366–368.

FUKS, Ž., LANMAN, R. C. & SCHANKER, L. S. (1964). *Int. J. Neuropharmac.*, **3**, 623–633.

FUXE, K., FARNEBO, L. O., HAMBERGER, B. & ÖGREN, S. O. (1975). *Postgrad. med. J.*, **51**, *Suppl. I*, 35–45.

GARATTINI, S., DE GAETANO, G., SAMANIN, R., BERNASCONI, S. & RONCAGLIONI, M.C. (1976). *Biochem. Pharmac.*, **25**, 13–16.

PRAGA, C. A. & POGLIANI, E. M. (1973). *Thromb. Diath. haemorrh.*, **29**, 183–189.

ROSS, S. B. & RENYI, A. L. (1975). *Acta pharmac. tox.*, **36**, 382–394.

STACEY, R. S. (1961). *Br. J. Pharmac.*, **16**, 284–295.

TUOMISTO, J. (1974). *J. Pharm. Pharmac.*, **26**, 92–100.

WIELOSZ, M., SALMONA, M., DE GAETANO, G. & GARATTINI, S. (1976). *Naunyn-Schmiedebergs Arch. Pharmac.*, in the press.

WONG, D. T., BYMASTER, F. P., HORNG, J. S. & MOLLOY, B. B. (1975). *J. Pharmac. exp. Ther.*, **193**, 804–811.

YATES, C. M., TODRICK, A. & TAIT, A. C. (1964). *J. Pharm. Pharmac.*, **16**, 460–463.

A new g.c. procedure, based on nitrosation, for the simultaneous determination of propoxyphene and norpropoxyphene in biological material

WILLEM J. SERFONTEIN*, LOUIS S. DE VILLIERS, *Department of Chemical Pathology, University of Pretoria, P. O. Box 2034, Pretoria, South Africa*

Propoxyphene is a widely used oral analgesic which is metabolized in man mainly to the secondary amine norpropoxyphene (Lee, Scott & Pohland, 1959) and to a lesser extent to the primary amine dinorpropoxyphene (McMahon, Sullivan & others, 1973).

We present a method in which norpropoxyphene is converted into *N*-nitrosonorpropoxyphene by reaction at low temperature with sodium nitrite. Under these conditions propoxyphene does not react appreciably; norpropoxyphene is converted almost quantitatively into *N*-nitrosonorpropoxyphene thus permitting the extraction of both compounds from an acidic medium into small volumes of chloroform. At the same time the primary amine dinorpropoxyphene may be expected to be converted into an alcohol with different chromatographic properties. This selective concentration thus permits and eliminates most other basic contaminants which contribute to the background on the gas chromatograms.

Dextropropoxyphene hydrochloride and (+)-norpropoxyphene hemicitrate (Eli Lilly Inc.) were used. The impurities normally present in samples of norpropoxyphene (Verebely & Inturissi, 1973) were shown to be carried through the extraction with norpropoxyphene itself but were satisfactorily separated at the chromatographic stage. Solvents were acid and alkali washed and distilled in an all-glass apparatus. All glassware was silylized in the usual manner.

Standard solutions were prepared by adding 0–5 μg of propoxyphene and 0–20 μg of norpropoxyphene to 2 ml of urine and 0–5 μg each of both drugs to 10 ml of serum. Calibration curves were constructed using cocaine (10 mg per 100 ml ethanol) as an internal standard. 70 μl of this solution were added to urine samples and 50 μl to serum samples at the beginning of the analysis.

Blood samples were centrifuged within 1–2 h of collection and the serum stored at —15° until analysed. Urine samples were similarly stored at —15°.

A Packard Model 824 gas chromatograph fitted with dual F.I.D. and silylized 0·5 m glass columns (3 mm internal diameter) packed with 1% SE 30 on

* Correspondence.