Biochemical Pharmacology, Vol. 26, pp. 647–649. Pergamon Press, 1977. Printed in Great Britain.

# Effects of methoxyamphetamines on the uptake and release of [$^3$H]5-hydroxytryptamine by human blood platelets

(*Received* 26 *March* 1976; *accepted* 3 *September* 1976)

We have recently demonstrated that *para*-methoxyamphetamine (4-MA) a known hallucinogen, is the most potent compound among monomethoxyamphetamines in inhibiting uptake and increasing the release of [$^3$H]5-hydroxytryptamine ([$^3$H]5-HT) in rat brain slices [1]. The correlation between the psychotomimetic potencies [2–4] and their effects on 5-HT neurones led us to suggest that 5-HT might be involved in the production of the psychotomimetic effect of 4-MA [1, 5].

The purpose of this study was to determine the effects of 4-MA and other methoxylated amphetamines on the uptake and release of 5-HT by platelets, a cell similar to the central serotonergic neurone [6, 7]. It is hoped that greater insight may be gained regarding the mechanism of action of methoxyamphetamines by developing a simple model system.

Fresh platelet-rich human plasma was prepared by Irwin Memorial Blood Bank of the San Francisco Medical Society, San Francisco, CA, and was used on the same day.

To study the effect of methoxyamphetamines on [$^3$H]5-HT uptake, 0.5 ml of platelet-rich plasma was diluted with 1.5 ml of calcium-free modified Tyrode solution as described previously [8] and was preincubated for 5 min at 37° in the presence of various concentrations of methoxyamphetamines. Subsequently, the platelets were incubated for another 2 min in the presence of [$^3$H]5-HT ($1 \times 10^{-7}$ M). The reaction was terminated by the addition of 5 ml of ice-cold modified Tyrode followed by rapid filtration on a modified Buchner funnel using Whatman glass fiber paper (GF/C). After washing twice with 5 ml of cold modified Tyrode solution, the platelets with filter paper were dissolved in 10 ml of Scinti Verse (Fisher Scientific Co.) in counting vials. The radioactivity was determined using liquid scintillation spectrometry.

To study the release of [$^3$H]5-HT from platelets, platelet-rich plasma, 0.25 ml, was diluted with equal volume of modified Tyrode solution and was incubated with [$^3$H]5-HT ($1 \times 10^{-7}$ M) for 20 min. After incubation, the platelets were centrifuged for 20 min at 2000 *g* and the supernatant was discarded. The platelets were washed twice with modified Tyrode solution and finally the platelets were suspended in 2 ml of modified Tyrode solution and incubated in the presence of methoxyamphetamine for 20 min. The radioactivity remaining in the platelets was then determined by the same procedure as in the uptake study. Release induced by the methoxyamphetamines was then calculated from the difference between samples incubated with and without addition of the drugs under the same experimental conditions.

The partition coefficient of each methoxyamphetamine was determined in duplicate at room temperature (22°) using *n*-heptane and 0.1 M phosphate buffer, pH 7.4. Both the *n*-heptane and buffer were saturated with the relevant aqueous or organic phase before use. Equal volumes (3 ml) of both phases were used and 2-hr agitation was allowed to achieve equilibrium. The initial concentration and final concentration before and after the equilibrium in the aqueous phase was then measured by using a DU spectrophotometer (nm = 280).

Racemates of methoxyamphetamines were purchased from Fox Chemical Co., Los Angeles, CA. *d-Para*-methoxyamphetamine HCl (4-d-MA) and l-*para*-methoxyamphetamine HCl (4-l-MA) were obtained from Dr. C. F. Barfknecht, College of Pharmacy, The University of Iowa, Iowa City, IA. d-Amphetamine $SO_4$ was purchased from Sigma Chemical Co., St. Louis, MO. 5-[1,2-$^3$H]-Hydroxytryptamine binoxalate ([$^3$H]5-HT, 4.25 mCi/m-mole) was purchased from New England Nuclear, Boston, MA. Chlorimipramine was a gift from Ciba Pharmaceutical Co., Summit, NJ.

The concentrations of methoxyamphetamines, amphetamines and chlorimipramine that cause 50 per cent inhibition of uptake ($IC_{50}$) of [$^3$H]5-HT and the potency ratios by using *d*-amphetamine as a reference compound (potency ratio = 1) are shown in Table 1. Chlorimipramine was found to be about 10,000 times more potent than *d*-amphetamine in inhibiting the uptake of [$^3$H]5-HT into the platelets. Among monomethoxyamphetamines, 4-d-MA was found to be the most potent compound (potency ratio = 21.6) followed in decreasing order of potency by 4-dl-MA, 4-l-MA, *dl*-meta-methoxyamphetamine (3-dl-MA) and *dl-ortho*-methoxyamphetamine (2-dl-MA). *Dextro*-isomers of 4-MA and amphetamine were more potent than the respective *levo*-isomers. Where comparisons were made among dimethoxyamphetamines and trimethoxyamphetamines, 3,4-*dl*-dimethoxyamphetamine (3,4-dl-DMA) was the most potent compound (potency ratio = 5.9) followed in decreasing order of potency by 3,5-dl-DMA, 2,4-dl-DMA, 2,3-dl-DMA, 2,5-dl-DMA, and 2,6-dl-DMA. 2,3,4-*dl*-Trimethoxyamphetamine (2,3,4-dl-TMA) and 2,4,5-dl-TMA were the most potent compounds among trimethoxyamphetamines followed in decreasing order of potency by 2,4,6-dl-TMA, 2,3,5-dl-TMA and 2,3,6-dl-TMA. In general, the activity of methoxyamphetamines decreased by increasing the number of methoxyl groups. The double reciprocal plot shown in Fig. 1 indicated that 4-d-MA and 4-l-MA competitively inhibited 5-HT uptake into platelets. The inhibitory constant ($K_i$) for 4-d-MA and 4-l-MA was found to be $8.3 \times 10^{-7}$ M and $5.0 \times 10^{-6}$ M respectively.

Among monomethoxyamphetamines, 4-d-MA was found to be the most active compound followed by 3-dl-MA and 2-dl-MA in increasing the release of [$^3$H]5-HT from platelets (Table 2). 4-d-MA was more active than 4-l-MA. Dimethoxyamphetamines are in general less active than monomethoxyamphetamines and a higher dose ($1 \times 10^{-4}$ M) was needed to induce the release. Trimethoxyamphetamines were found to be less active than di- and monomethoxyamphetamine. In general, the potencies of methoxyamphetamines in increasing the release of [$^3$H]5-HT are roughly parallel with that of inhibition of uptake of [$^3$H]5-HT in platelets.

In contrast to the effects of 4-MA which is effective in both inhibiting the uptake and increasing the release of [$^3$H]5-HT by platelets, chlorimipramine was very potent in inhibiting the uptake of [$^3$H]5-HT into the platelets but was only about 10 times more potent than *d*-amphetamine in increasing the release of [$^3$H]5-HT. This is consistent with the lack of hallucinogenic properties of chlorimipramine.

Our results indicated that *para*-methoxylation of amphetamine increased and *ortho*-methoxylation of amphetamine reduced the effects of the analogues on blocking the uptake and increasing the release of [$^3$H]5-HT in platelets. *Meta-*

Table 1. Effects of amphetamines and methoxyamphetamines on the uptake of [$^3$H]5-HT into the blood platelets*

| | | $IC_{50}$ (M) | Potency ratio (*d*-amphetamine = 1) | Partition coefficient $K$† | MU | |
|---|---|---|---|---|---|---|
| | | | | | Man | Rat |
| A | Chlorimipramine | $2.98 \pm 0.37 \times 10^{-9}$ (4) | 10872 | | | |
| B | Amphetamine | | | | | |
| | *d*-Amphetamine | $3.24 \pm 1.19 \times 10^{-5}$ (5) | 1 | 0.14 | | |
| | *l*-Amphetamine | $1..24 \pm 0.20 \times 10^{-4}$ (6) | 0.3 | | | |
| C | Monomethoxyamphetamine | | | | | |
| | 4-d-MA | $1.50 \pm 0.20 \times 10^{-6}$ (4) | 21.6 | | | 16 |
| | 4-l-MA | $6.80 \pm 1.42 \times 10^{-6}$ (3) | 4.7 | | | |
| | 4-dl-MA | $2.18 \pm 0.58 \times 10^{-6}$ (5) | 14.9 | 0.44 | 5 | |
| | 3-dl-MA | $1.44 \pm 0.26 \times 10^{-5}$ (5) | 2.3 | 0.47 | | 1.5 |
| | 2-dl-MA | $1.19 \pm 0.24 \times 10^{-4}$ (5) | 0.3 | 0.30 | | 1 |
| D | Dimethoxyamphetamine | | | | | |
| | 3,4-dl-DMA | $5.47 \pm 1.21 \times 10^{-6}$ (6) | 5.9 | | 1 | 1.6 |
| | 3,5-dl-DMA | $1.85 \pm 0.20 \times 10^{-5}$ (6) | 1.8 | | | |
| | 2,4-dl-DMA | $1.92 \pm 0.43 \times 10^{-5}$ (6) | 1.7 | | 5 | 5.6 |
| | 2,3-dl-DMA | $4.12 \pm 0.50 \times 10^{-5}$ (5) | 0.8 | | | |
| | 2,5-dl-DMA | $1.28 \pm 0.34 \times 10^{-4}$ (5) | 0.3 | 0.35 | 8 | 2.2 |
| | 2,6-dl-DMA | $2.73 \pm 0.20 \times 10^{-4}$ (4) | 0.1 | 0.38 | | |
| E | Trimethoxyamphetamine | | | | | |
| | 2,3,4-dl-TMA | $2.57 \pm 0.20 \times 10^{-5}$ (3) | 1.3 | 0.18 | 2 | 1.8 |
| | 2,4,5-dl-TMA | $2.88 \pm 0.18 \times 10^{-5}$ (4) | 1.1 | | 17 | 8.2 |
| | 2,4,6-dl-TMA | $4.73 \pm 0.90 \times 10^{-5}$ (4) | 0.7 | | 10 | 4.5 |
| | 2,3,5-dl-TMA | $1.16 \pm 0.16 \times 10^{-4}$ (4) | 0.3 | 0.15 | 4 | 1.4 |
| | 2,3,6-dl-TMA | $3.53 \pm 0.24 \times 10^{-4}$ (4) | 0.1 | 0.08 | 13 | 1.2 |

* Blood platelets were incubated with $1 \times 10^{-7}$ M [$^3$H]5-HT in the presence of various concentrations of drugs. The $IC_{50}$ values were determined by graphical analysis and each value represents the mean ±S. E. with the number of determinations indicated in parentheses. For comparing the potency, the $IC_{50}$ of *d*-amphetamine was used as 1 MU (mescaline unit); the data are cited from Refs. 2 and 4.

† $K$ partition coeffieient = concentration of drug in *n*-heptane/concentration of drug in 0.1 M phosphate buffer.

methoxylation of amphetamine altered slightly the ability of these compounds to block the uptake and increase the release of [$^3$H]5-HT.

To test the possibility that the different potencies of methoxyamphetamines on the uptake inhibition and increased release of [$^3$H]5-HT by platelets were due to the differences in lipid solubility, the partition coefficients of those compounds were determined. As shown in Table 1, monomethoxyamphetamines and di-methoxyamphetamines were found to be more lipid soluble than *d*-amphetamine and trimethoxyamphetamines. However, there was no correlation between the partition coefficients of methoxyamphetamine and their potencies in inhibiting the uptake of [$^3$H]5-HT by platelets ($\Omega = 0.47$, $P > 0.05$). Thus, the different potencies of methoxyamphetamine on the 5-HT of platelets cannot be explained based on the different rate of diffusional process across the cell membrane.

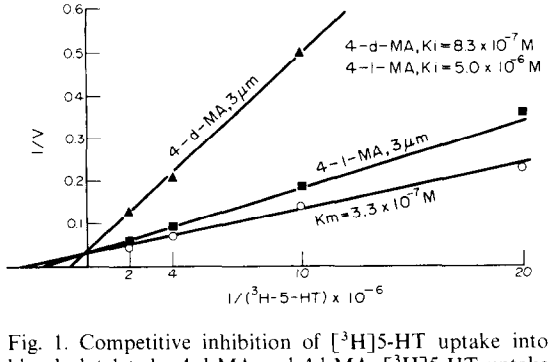


Fig. 1. Competitive inhibition of [$^3$H]5-HT uptake into blood platelets by 4-d-MA and 4-l-MA. [$^3$H]5-HT uptake ($V$) is given as pmoles/0.5 ml of plasma/2 min. N = 4/point. Key: (○) control; (▲) 4-d-MA; and (■) 4-l-MA.

There was an impressive correlation between the psychotomimetic potencies and the effects on blocking the uptake and increasing the release of [$^3$H]5-HT of the monomethoxyamphetamines, whereas no such correlation existed with the polymethoxyamphetamines. Harris *et al.* [4] and Smythies *et al.* [3] reported that 4-MA was the most potent compound in disrupting the operant behavior in rats, followed by 3- and 2-MA. Similarly, the serotonergic myoclonic twitch activity of suprahyoideal muscle in rats was activated by the monomethoxyamphetamines with the same order of potencies [5]. This same order of potency was observed with blocking the uptake and increasing the release of [$^3$H]5-HT by platelets and by rat brain slices in a previous study [1]. This correlation suggests that 4-MA elicits its psychotomimetic effect by altering presynaptic 5-HT neuronal activity.

However, dimethoxyamphetamines and trimethoxyamphetamines were found not to be active in inhibiting the uptake and increasing the release of [$^3$H]5-HT, though some of them were reported to be potent hallucinogens (see Table 1). Obviously, mechanisms other than the effects involving the release and uptake of 5-HT at pre-synaptic 5-HT neurons must be considered for the action of dimethoxyamphetamine and trimethoxyamphetamines. One possible mechanism is that those methoxyamphetamines may act directly at 5-HT receptors. In support of this possibility, Wallach *et al.* [9] proposed that 2,5-dimethoxy-4-methylamphetamine (DOM) induces its behavioral effects by acting at post-synaptic sites, since the behavior syndrome induced by DOM is not influenced by the pre-treatment with *para*-chlorphenylalanine. Cheng *et al.* [10] reported that DOM activates 5-HT receptors in vascular strips of dog dorsal metatarsal vein. Studies are in progress to verify this possibility.

Table 2. Effects of amphetamines and methoxyamphetamines on the release of [$^3$H]5-HT from human blood platelets*

| | | $1 \times 10^{-6}$ M | $1 \times 10^{-5}$ M | $1 \times 10^{-4}$ M |
|---|---|---|---|---|
| A | Chlorimipramine | 10.65 ± 3.97 | 29.67 ± 4.21 | |
| B | Amphetamine | | | |
| | *d*-Amphetamine | 1.20 ± 6.54 | 9.00 ± 5.50 | 29.54 ± 3.27 |
| | *l*-Amphetamine | 0.77 ± 4.38 | −3.15 ± 2.28 | 13.23 ± 6.93 |
| C | Monomethoxyamphetamine | | | |
| | 4-d-MA | 6.08 ± 2.39 | 21.73 ± 4.55 | |
| | 4-l-MA | 6.91 ± 2.98 | 9.93 ± 3.22 | |
| | 4-dl-MA | 0.38 ± 2.88 | 19.34 ± 3.66 | 28.19 ± 0.81 |
| | 3-dl-MA | | 9.01 ± 4.74 | 34.08 ± 9.81 |
| | 2-dl-MA | | 3.25 ± 1.88 | 16.34 ± 2.24 |
| D | Dimethoxyamphetamine | | | |
| | 3,4-dl-DMA | −1.17 ± 2.47 | 1.95 ± 3.73 | 10.01 ± 1.37 |
| | 3,5-dl-DMA | −0.54 ± 1.49 | 2.24 ± 0.83 | 14.31 ± 1.79 |
| | 2,4-dl-DMA | −3.25 ± 6.88 | 0.08 ± 1.81 | 16.66 ± 4.35 |
| | 2,3-dl-DMA | | 3.65 ± 1.34 | 13.01 ± 2.53 |
| | 2,5-dl-DMA | | 0.95 ± 2.36 | 5.91 ± 1.77 |
| | 2,6-dl-DMA | | 0.96 ± 2.81 | 8.61 ± 3.33 |
| E | Trimethoxyamphetamine | | | |
| | 2,3,4-dl-TMA | | 4.22 ± 2.46 | 7.83 ± 4.95 |
| | 2,4,5-dl-TMA | | −7.29 ± 8.87 | 9.12 ± 2.65 |
| | 2,4,6-dl-TMA | | −21.19 ± 7.66 | −2.21 ± 2.62 |
| | 2,3,5-dl-TMA | | −1.73 ± 4.98 | −0.32 ± 11.68 |
| | 2,3,6-dl-TMA | | −3.06 ± 4.35 | −0.24 ± 3.49 |

* Blood platelets were preincubated with [$^3$H]5-HT ($1 \times 10^{-7}$ M). After suitable washings, the effect of drugs on the release of [$^3$H]5-HT was measured. The data are expressed as

$$100 - \frac{\text{radioactivity of blood platelets with drug}}{\text{radioactivity of blood platelets without drug}} \times 100.$$

Each value is the mean ± S. E. of four to five determinations.

*Acknowledgements*—These studies were supported by NIDA Grant DA-01314 (L.-F. T.) and NIMH Grant MH-25487 (H. H. L.), and a grant from the Academic Senate, University of California, San Francisco. The authors express their gratitude to Dr. David Johnson for helpful editorial suggestions, Miss Virginia Pascucci for able technical assistance, and Ms. Virginia Hayes for typing assistance.

*Departments of Psychiatry and Pharmacology,*
*University of California,*
*San Francisco, CA* 94143, *U.S.A.*

LIANG-FU TSENG
HORACE H. LOH

## REFERENCES

1. L. F. Tseng, M. K. Menon and H. H. Loh, *J. Pharmac. exp. Ther.* **197,** 263 (1976).
2. A. T. Shulgin, T. Sargent and C. Naranjo, *Nature, Lond.* **221,** 537 (1969).
3. J. R. Smythies, V. S. Johnston, R. J. Bradley, F. Benington, R. D. Morin and L. C. Clark, *Nature, Lond.* **216,** 138 (1967).
4. R. A. Harris, D. Snell, H. H. Loh and C. D. Leake, *Fedn Proc.* **35,** 2421 (1976).
5. M. K. Menon, L. F. Tseng and H. H. Loh, *J. Pharmac. exp. Ther.* **197,** 272 (1976).
6. M. K. Paasonen, *Annls Med. exp. Biol. Fenn.* **46,** 416 (1968).
7. M. K. Paasonen, *Proc. Fifth Int. Cong. Pharmac.* (Eds. F. F. Bloom and G. H. Acheson), Vol. 4, p. 328. S. Karger, Basel (1973).
8. M. DaPrada, G. Bartholini and A. Pletsher, *Biochem. Pharmac.* **14,** 1721 (1965).
9. M. B. Wallach, E. Friedman and S. Gershon, *J. Pharmac. exp. Ther.* **182,** 145 (1972).
10. H. C. Cheng, J. P. Long, D. E. Nichols and C. F. Barfkneckt, *J. Pharmac. exp. Ther.* **188,** 114 (1974).