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Effects of serotonin uptake inhibitor, Lilly 110140, on transport of serotonin in rat and human blood platelets*

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The uptake of serotonin (5HT) into blood platelets is a substrate saturable process with K_m value of 2.3×10^{-7} M and dependent upon metabolic energy, temperature and sodium [1, 2]. Tricyclic antidepressants, imipramine and chlorimipramine are known to inhibit the process [3]. The process for uptake of 5HT precedes the storage of the accumulated amine which can be depleted by the treatment of reserpine [4]. Platelets, therefore, have been extensively used by investigators as a model for the study of similar processes that occur in central serotoninergic neurons [5, 6].

3-(*p*-Trifluoromethylphenoxy)-*N*-methyl-3-phenylpropylamine hydrochloride (Lilly 110140) has been reported as a specific inhibitor of 5HT uptake into serotoninergic nerve terminals of rat brain *in vitro* [7, 8] as well as *in vivo* [9]. The present report shows that Lilly 110140 also inhibits the process of 5HT uptake in platelets of rat and human blood plasma.*

Male albino Wistar-derived rats weighing 300 g were supplied by Harlan Industries, Cumberland, Indiana. Platelet-rich plasma (PRP) was prepared according to the described method [1].

For the measurement of [^{14}C]5HT uptake into platelets *in vitro*, aliquots of PRP in 0.2 ml were mixed with 0.8 ml Krebs bicarbonate medium containing ascorbic acid and 1.25×10^{-7} M [^{14}C]5HT, and were incubated at 37° for 10 min. Platelets were spun down by centrifugation at 4340 *g* for 10 min at 4°; the sediment accounted for 97 per cent of platelets in PRP. Platelets were digested in 0.2 ml of 30% H_2O_2 and transferred to counting vials containing 10 ml of scintillation fluid (Permafluor: Triton X-100: Toluene with a proportion of 1:8:16). The radioactivity was measured with a Packard liquid scintillation spectrometer. Proteins were determined by the biuret method [10].

To demonstrate the effects of drugs *in vivo*, rats were treated twice with either Lilly 110140, chlorimipramine or desipramine at 10 mg/kg i.p. 24 and 0.5 hr before an i.v. injection of [^{3}H]5HT (10 μC/kg). The rats were sacrificed 30 min after the administration of [^{3}H]5HT. Blood, PRP and platelets were collected as previously described except that the separation of PRP from whole blood was carrried out at 4°. The radioactivity in platelets and platelet-free plasma were measured. At least 70 per cent of the total radioactivity in blood platelets of rats was identified as 5HT by the method described by Schubert *et al.* [11].

Human blood was obtained from healthy donors by venous puncture with a 20-ml plastic syringe containing 2 ml of 3.8% citrate. The blood was centrifuged at 60 *g* for 15 min at 4° and PRP was pipetted off. An aliquot of PRP (0.5 ml) was mixed with 0.5 ml of Krebs bicarbonate medium containing 2×10^{-7} M [^{14}C]5HT. Active uptake of [^{14}C]5HT was measured as previously described except that platelets were separated by a centrifugation at 12,100 *g* for 20 min.

Chemicals. 3-(*p*-Trifluoromethylphenoxy)-*N*-methyl-3-phenylpropylamine hydrochloride (Lilly 110140) and its *N*-unsubstituted amine and *N*,*N*-dimethylamino analogs were synthesized by Dr. B. B. Molloy of the Lilly Research Laboratories. All drugs were used as aqueous solutions of their hydrochloride salts. [2-^{14}C]5HT, 25 mCi/m-mole; and [1,2-^{3}H]*N*-5HT, 1 mCi/0.68 μmole were purchased from New England Nuclear. Tricyclic compounds: imipramine, chlorimipramine and desipramine were obtained from Ciba Geigy Corp., Ardsley, New York.

The inhibition curves of [^{14}C]5HT uptake into rat blood platelets by tricyclic antidepressant drugs, Lilly 110140 and amphetamine, are shown in Fig. 1. In inhibition of [^{14}C]5HT uptake into blood platelets, chlorimipramine was most potent, followed by Lilly 110140 and imipramine with IC_{50} values of 4.0×10^{-8} M, 1×10^{-7} M and 1.6×10^{-7} M, respectively. All three drugs produced complete inhibition of [^{14}C]5HT uptake at approximately the same concentration (5×10^{-6} M). The secondary amine containing tricyclic compounds, nortriptyline and desipramine, were less potent for inhibiting [^{14}C]5HT with IC_{50} values of 3.8×10^{-7} and 6×10^{-7} M, respectively, while amphetamine was the least active with an IC_{50} value of 2.2×10^{-5} M.

The effects of the tricyclic drugs and Lilly 110140 on the uptake of [^{3}H]5HT into platelets *in vivo* were examined. Lilly 110140 and chlorimipramine inhibited the uptake of 5HT into platelets by 63 per cent ($P < 0.001$) and 53 per cent ($P < 0.005$), respectively (Fig. 2A). A 28 per cent inhibition was also caused by desipramine but was not statistically significant. A high ratio of [^{3}H]5HT in platelets to that of in platelet-free plasma indicated active uptake of [^{3}H]5HT (Fig. 2B). The administration of Lilly 110140, chlorimipramine and desipramine, signifi-

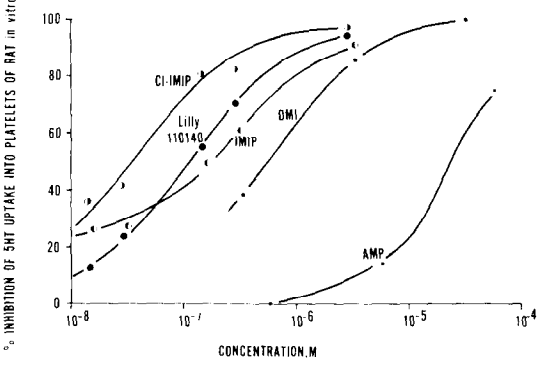

Fig. 1. The effects of Lilly 110140, amphetamine and tricyclic antidepressants on the uptake of [^{14}C]5HT into rat blood platelets *in vitro*. [^{14}C]5HT at 0.1 μM was included in the medium and other experimental conditions were the same as described in the text. Abbreviations used: Cl-IMIP, chlorimipramine; IMIP, imipramine; DMI, desipramine and AMP, amphetamine.

* This work was presented in part at the 58th Annual Meeting of the Federation of American Societies for Experimental Biology, April 7–12, 1974.

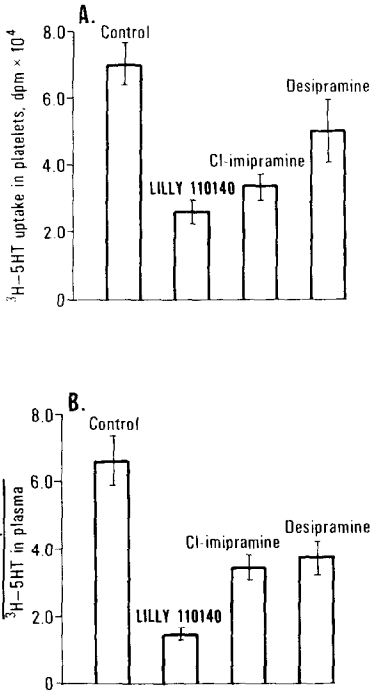

Fig. 2. The effects of Lilly 110140, chlorimipramine and desipramine on [^{3}H]5HT uptake into platelets (A) and [^{3}H]5HT in platelets/[^{3}H]5HT in platelet-free plasma, ratios (B) *in vivo*. Rats twice treated with either Lilly 110140, chlorimipramine or desipramine at 10 mg/kg i.p. within 24 hr, [^{3}H]5HT (10 μC/kg) was injected i.v. 30 min after the last dose of the drug and were sacrificed 30 min thereafter. PRP was obtained by a centrifugation at 100 *g* for 40 min at 4°. The platelets and platelet-free plasma (PFP) were further separated by centrifugation at 4340 *g* for 10 min. Radioactivity of digested platelets and PFP was monitored by scintillation technique.

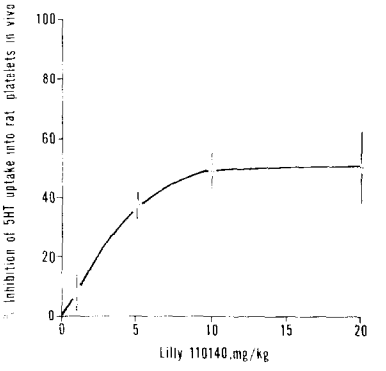

Fig. 4. The dose response curve of Lilly 110140 inhibiting the uptake of [^{3}H]5HT into rat platelets *in vivo*. Experimental conditions were described in Fig. 2 except that a single dose of Lilly 110140 at various levels was i.p. injected 30 min prior to the i.v. administration of [^{3}H]5HT (6 μCi/kg). Animals were sacrificed 15 min thereafter. Mean rate of [^{3}H]5HT uptake into platelets of 9 saline-treated rats was 52.28 ± 2.97 nCi/ml PRP.

cantly reduced the ratio from a control value of 6.6 to 1.5, 3.5 and 3.8, respectively.

The time-courses of inhibition of [^{3}H]5HT uptake into platelets *in vivo* after the administration of Lilly 110140 and chlorimipramine are compared in Fig. 3. Lilly 110140 (10 mg/kg i.p.) inhibited over 60 per cent of [^{3}H]5HT uptake into platelets after 30 min of drug administration and remained effective for a period of at least 12 hr. A lesser degree of inhibition (40 per cent) was found by an equimolar dose of chlorimipramine, and the inhibition only lasted for a 4-hr period.

Fig. 4 shows the dose response of Lilly 110140 on the uptake of [^{3}H]5HT into rat platelets *in vivo*. Lilly 110140 at 10 mg/kg i.p. exerted a maximum inhibition on [^{3}H]5HT uptake. A further increase in the dose of Lilly

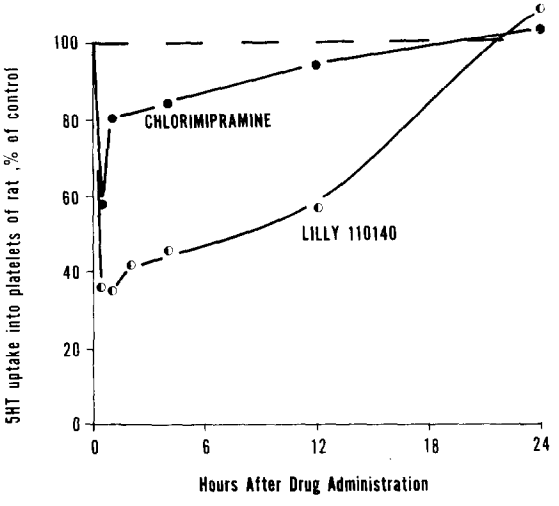

Fig. 3. The comparison in duration of the effect of Lilly 110140 and chlorimipramine on [^{3}H]5HT uptake into platelets *in vivo*. The experimental conditions were the same as in Fig. 2 except that a single dose of either drug at 10 mg/kg i.p. was used. Mean rate of [^{3}H]5HT uptake into platelets of 13 saline-treated rats was 20.89 ± 1.33 nCi/ml PRP.

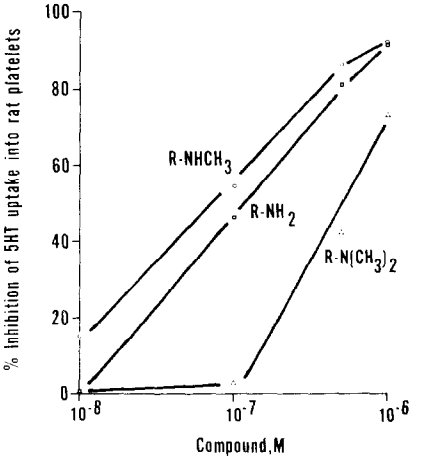

Fig. 5. The effects of Lilly 110140, the *N*-unsubstituted and the *N,N*-dimethylated analogs on the uptake of [^{14}C]5HT into rat platelets *in vitro*. The experimental conditions were the same as described in Fig. 1. Abbreviations used: R-NHCH$_3$, Lilly 110140; R-N(CH$_3$)$_2$, *N,N*-dimethylated compound and R-NH$_2$, the *N*-unsubstituted compound. Mean value of 5HT uptake into rat platelets was 52.42 ± 0.80 pmole/ml PRP/min.

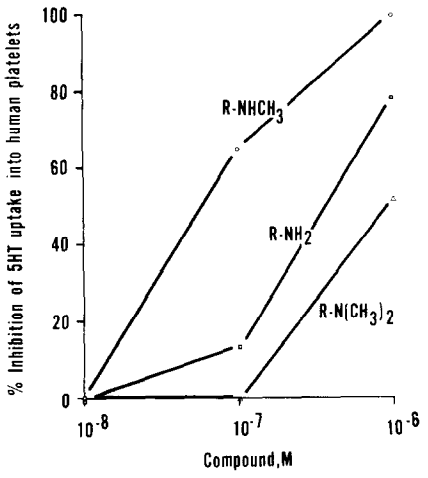

Fig. 6. The effects of Lilly 110140, the *N*-unsubstituted and the *N,N*-dimethylated analogs on uptake of [^{14}C]5HT into human platelets *in vitro*. [^{14}C]5HT at 0.1 μM was used as substrate. Other experimental conditions were described in the text. Abbreviations were identical to those used in Fig. 5. Mean value of 5HT uptake into human platelets was 6.61 $\pm$ 0.22 pmole/ml PRP/min.

110140 to 20 mg/kg did not produce a greater degree of inhibition.

Fig. 5 shows the effects of Lilly 110140 (R-NHCH$_3$), its *N*-unsubstituted primary amine and *N,N*-dimethyl tertiary amine subsituted analogs on the uptake of [^{14}C]5HT into rat blood platelets *in vitro*. The *N*-unsubstituted analog of Lilly 110140 was about as active as Lilly 110140 in inhibition of 5HT uptake into platelets while the *N,N*-dimethyl substituted analog was not as active having an IC_{50} value of 6×10^{-7} M. The effects of the three compounds on the uptake of [^{14}C]5HT into human platelets *in vitro* were also compared (Fig. 6). The unsubstituted and the *N,N*-dimethyl substituted analogs were not as active as Lilly 110140 in inhibition of [^{14}C]5HT uptake into human platelets, having IC_{50} values of 3.7×10^{-7} M, 1×10^{-6} M and 5.4×10^{-8} M, respectively.

Like the tertiary amine containing tricyclic antidepressants, chlorimipramine and imipramine, Lilly 110140 is also a potent inhibitor of 5HT uptake into rat platelets both *in vitro* and *in vivo*. This is in agreement with the effect of Lilly 110140 in brain tissues in which selectivity of Lilly 110140 toward the uptake sites for 5HT over NE has been demonstrated [7, 8, 9]. Unlike the tricyclic antidepressants, however, Lilly 110140 has no effect on the uptake of NE into rat heart [12, 13].

The short duration and the lack of potency of chlorimipramine on [^{3}H]5HT uptake into platelets *in vivo* may reflect the *N*-demethylation of chlorimipramine, giving chlorodesipramine, which has been shown in our laboratory to be a weaker inhibitor of 5HT uptake but a better inhibitor for NE uptake. *N*-Demethylation of Lilly 110140 also occurs, and the *N*-demethylated product persists in circulation for longer than 30 hr [14]. In contrast to chlorimipramine, however, the *N*-demethylated product of Lilly 110140 as seen in the present study is equally active in inhibition of 5HT uptake into rat blood platelets. Thus both Lilly 110140 and its *N*-demethylated product might be responsible for the prolonged inhibition of 5HT uptake into platelets *in vivo*.

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REFERENCES

1. J. M. Sneddon, *Br. J. Pharmac.* **37**, 680 (1969).
2. M. K. Passonen, L. Shtee and E. Solatunturi, *Progr. Brain Res.* Vol. 34, p. 269 (1971).
3. E. F. Marshall, G. S. Stirling, A. C. Tait and T. Todrick, *Br. J. Pharmac.* **15**, 35 (1959).
4. A. Pletscher, W. P. Burkard, J. P. Tranzer and K. F. Gey, *Life Sci.* **6**, 273 (1967).
5. J. M. Sneddon, *Progr. Neurol.*, Vol. 1, pt. 2, p. 151, Pergamon Press, Oxford (1973).
6. J. Tuomisto, *J. Pharm. Pharmac.* **26**, 92 (1974).
7. D. T. Wong, F. P. Bymaster, J. S. Horng and B. B. Molloy, *Fedn. Proc.* **33**, 255 (1974).
8. D. T. Wong, J. S. Horng, F. P. Bymaster, K. L. Hauser and B. B. Molloy, *Life Sci.* **15**, 471 (1974).
9. R. W. Fuller and K. W. Perry, *Fedn Proc.* **33**, 255 (1974).
10. A. G. Gornall, C. J. Bardawell and M. M. David, *J. biol. Chem.* **177**, 751 (1949).
11. J. Schubert, H. Nyback and G. Sedvall, *Eur. J. Pharmac.* **10**, 215 (1970).
12. J. S. Horng, F. P. Bymaster and D. T. Wong, *Fedn Proc.* **33**, 560 (1974).
13. K. W. Perry and R. W. Fuller, *Fedn Proc.* **33**, 560 (1974).
14. C. J. Parli and J. Hicks, *Fedn Proc.* **33**, 560 (1974).