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UPTAKE AND RELEASE OF 5-HYDROXYTRYPTAMINE BY ENTERIC 5-HYDROXYTRYPTAMINERGIC NEURONES: EFFECTS OF FLUOXETINE (LILLY 110140) AND CHLORIMIPRAMINE

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The effect of fluoxetine on uptake of 5-hydroxytryptamine (5-HT) by enteric 5-hydroxytryptaminergic neurones has been analyzed in order to compare further these neurones with 5-HT neurones of the CNS. In addition, the effects of fluoxetine and chlorimipramine on efflux of [^{3}H]-5-HT from the myenteric plexus were also evaluated. Fluoxetine was found to be a competitive inhibitor of 5-HT uptake by the myenteric plexus and was a more potent inhibitor of 5-HT uptake than was chlorimipramine. However, chlorimipramine enhanced the efflux of [^{3}H]-5-HT more than could be explained by inhibition of 5-HT uptake and, therefore, appears to have the additional action of releasing the amine. These observations, similar to those of others studying central neurones, support the view that enteric 5-HT neurones resemble those of the CNS and are a useful model for the evaluation of drugs.

Introduction 5-Hydroxytryptaminergic neurones have been found in the mammalian myenteric plexus (Gershon, Dreyfus, Pickel, Joh & Reis, 1977). These neurones share many properties with the central 5-hydroxytryptaminergic neurones of the raphe including a specific uptake mechanism for 5-hydroxytryptamine (5-HT; Gershon, Robinson & Ross, 1976), tryptophan hydroxylase that cross reacts with antibody prepared against the enzyme purified from raphe neurones (Gershon, *et al.*, 1977), and the presence of a specific 5-HT-binding protein (SBP; Jonakait, Tamir, Rapport & Gershon, 1977). If these peripheral neurones are very much like the central 5-HT neurones, their accessibility would make them extremely valuable test objects for the evaluation of drug effects on 5-HT neurones.

Fluoxetine (Lilly 110140) has been found to be a more specific inhibitor of 5-HT uptake into brain synaptosomes than tricyclic antidepressants, such as chlorimipramine (CMI; Wong, Horng, Bymaster, Hauser & Molloy, 1974). CMI inhibits the uptake of 5-HT by enteric 5-HT neurones (Gershon *et al.*, 1976) but although it is the most potent tricyclic in this regard, it is relatively non-specific. There is also evidence from brain that CMI not only inhibits uptake but may also facilitate the efflux of 5-HT from tissue (Ashkenazi, Holman & Vogt, 1973; Farnebo & Hamberger, 1974). In order to evaluate further the resemblance between central and peripheral 5-HT neurones, we have studied the effect of fluoxetine on the uptake of 5-HT by enteric 5-HT neurones, and have compared fluoxetine with CMI with respect to their effect on 5-HT efflux from the gut.

Methods Strips (91) of longitudinal muscle with adherent myenteric plexus (LM-MP strips) were dissected from the ilea of 36 male guinea-pigs (weighing 350 to 400 g) and allowed to equilibrate at 37°C for 30 min in Krebs solution. In experiments designed to test the effects of fluoxetine on uptake, the drug (0.1, 0.7, 3.0, 10 or 50 μM) was included during both the equilibration and subsequent incubation periods. Following equilibration, [^{3}H]-5-HT (0.9 μM; 4 Ci/mmol, New England Nuclear) was added, and the incubation was allowed to continue for 5 min. Tissues were then passed sequentially through a series of vials containing Krebs solution at 4°C. Radioactivity in each vial was counted by liquid scintillation. Radioactivity remaining in the tissue following washout was extracted overnight in 70% ethanol (Gershon & Altman, 1971).

In experiments designed to test the effects of CMI and fluoxetine on the rate of 5-HT efflux, tissues were pre-loaded with [^{3}H]-5-HT for 30 min, and CMI (50 μM) or fluoxetine (10 μM) were included in the washout solution, which was, in these experiments, maintained at 37°C.

Washout curves were constructed in which the percentage of the total radioactivity remaining in the tissue was plotted semi-logarithmically as a function of time. Several compartments of washout were apparent. The compartment of slowest washout could be described by a single exponential equation. Previous studies on the uptake of [^{3}H]-5-HT by enteric neurones (Gershon & Ross, 1966; Gershon & Altman, 1971) have shown that this compartment represents unmetabolized 5-HT exclusively located in the myenteric plexus. By extrapolating the curve of washout of this slowest compartment back to zero time, the amount of 5-HT originally present in this compartment could be determined. Uptake was expressed as

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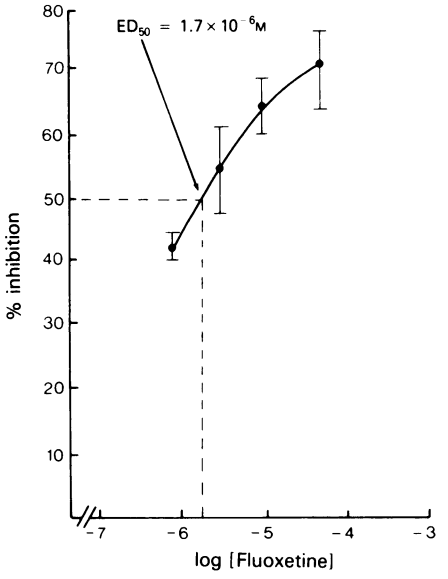

Figure 1 Concentration-effect curve showing the inhibition of [^{3}H]-5-hydroxytryptamine ([^{3}H]-5-HT) uptake by Lilly 110140 (fluoxetine). The ED_{50} shown on the curve was determined by log probit analysis (Goldstein, 1965). Each point is the mean of four determinations and the vertical bars represent s.e. mean.

d min^{-1} g^{-1} tissue divided by d min^{-1} ml^{-1} incubating solution, i.e. ml/g. Uptake which occurs by passive diffusion at 0°C was subtracted from uptake at 37°C to estimate active uptake. The half-time of washout ($T_{\frac{1}{2}}$) from the compartment of slowest washout was determined from the exponential equation describing the washout curve (Gershon & Ross, 1966; Gershon & Altman, 1971). Log probit analysis of concentration-effect curves was used to determine the ED_{50} and 95% confidence limits for drug inhibition of 5-HT uptake and Student's t test was used to compare sample means (Goldstein, 1965).

Results In the presence of fluoxetine, uptake of [^{3}H]-5-HT into neurones of the myenteric plexus was inhibited and this inhibition was concentration-dependent. Figure 1 shows the percentage inhibition of uptake plotted as a function of the logarithm of the fluoxetine concentration. The ED_{50} was determined from the curve to be 1.7 ± 0.38 μM (95% confidence limits).

The type of inhibition of 5-HT uptake by fluoxetine was studied by determining the effect of 1.7 μM fluoxetine on the V_{max} and K_m of 5-HT uptake. Noradrenaline (40 μM) was included during the incubation to insure that uptake into adrenergic nerve terminals was not occurring at high concentrations of 5-HT used in studying 5-HT uptake kinetics. The data were again corrected for uptake by diffusion which occurs at 0°C (Gershon & Altman, 1971), and then plotted according to the method of Lineweaver & Burk (1934). K_m for control uptake was 0.7 μM, and V_{max} was 0.4 nmol g^{-1} min^{-1} (Gershon, *et al.*, 1976). In the presence of fluoxetine, K_m was elevated (6.1 μM), but V_{max} was unchanged (0.44 nmol g^{-1} min^{-1}). The increase in K_m without a change in V_{max} implies that the inhibition of 5-HT uptake by fluoxetine was competitive.

When washouts of tissue preloaded with [^{3}H]-5-HT were performed at 37°C, the half-time of washout for the compartment of slowest washout was 43.6 ± 1.0 min (n = 7). When CMI (50 μM) was included during the washout period, the $T_{\frac{1}{2}}$ fell to 36% of control levels (15.8 ± 0.6 min; n = 4, $P < 0.001$). By contrast, washout in the presence of 10 μM fluoxetine resulted in a higher ($P < 0.001$) $T_{\frac{1}{2}}$ (26.0 ± 0.8 min; n = 12) although the $T_{\frac{1}{2}}$ in the presence of fluoxetine was still lower than control ($P < 0.001$).

Discussion These observations indicate that fluoxetine is a potent competitive inhibitor of 5-HT uptake by enteric 5-HT neurones. In fact, it is more potent than CMI (ED_{50}, 40 μM; Gershon *et al.*, 1976). However, the higher value for ED_{50} (1.7 μM) for fluoxetine in gut suggests that the drug is less potent in inhibiting intestinal than synaptosomal uptake (Ki = 52 nM; Wong *et al.*, 1974). This apparent difference in potency has also been observed for inhibition of gut 5-HT uptake by CMI (Gershon *et al.*, 1976). Since intact enteric neurones survive well and function *in vitro* for long periods of time, they are probably healthier under experimental conditions than synaptosomes which are neuronal fragments. This may account for the greater resistance of enteric than synaptosomal uptake of 5-HT to the effects of inhibitory drugs. Nevertheless, although there are apparent differences in potency between enteric and central systems, the two types of 5-HT neurones appear to be similarly affected by uptake inhibitors.

Both CMI and fluoxetine, by preventing re-uptake of [^{3}H]-5-HT were expected to and did enhance the rate of washout of the labelled amine. Since fluoxetine is a more potent inhibitor of 5-HT uptake than CMI at the concentrations used, it should have enhanced 5-HT efflux more than CMI if uptake inhibition were the only factor involved. However, CMI increased the rate of [^{3}H]-5-HT efflux more than did fluoxetine. Therefore, CMI must have an additional effect, that is, enhancement of 5-HT release. In this respect again, the enteric 5-HT neurones resemble their central counterparts (Ashkenazi *et al.*, 1973; Farnebo & Hamberger, 1974). These experiments support the hypothesis that peripheral 5-HT neurones are very similar to those of the CNS and are thus a valuable model for drug evaluation.

References

ASHKENAZI, R., HOLMAN, R.B. & VOGT, M. (1973). Release of transmitters into the perfused third ventricle of the cat. *J. Physiol.*, **233,** 195–209.

FARNEBO, L-O., & HAMBERGER, B. (1974). Regulation of [^{3}H]-5-hydroxytryptamine release from rat brain slices. *J. Pharm. Pharmac.*, **26,** 642–644.

GERSHON, M.D. & ALTMAN, R.F. (1971). An analysis of the uptake of 5-hydroxytryptamine by the myenteric plexus of the small intestine of the guinea pig. *J. Pharmac. exp. Ther.*, **179,** 29–41.

GERSHON, M.D., DREYFUS, C.F., PICKEL, V.M., JOH, T.H. & REIS, D.J. (1977). Serotonergic neurones in the peripheral nervous system. *Proc. natn. Acad. Sci. U.S.A.*, **74,** 3086–3089.

GERSHON, M.D., ROBINSON, R.G. & ROSS, L.L. (1976). Serotonin accumulation in the guinea-pig myenteric plexus: Ion dependence, structure-activity relationship and the effect of drugs. *J. Pharmac. exp. Ther.*, **198,** 548–561.

GERSHON, M.D. & ROSS, L.L. (1966). Location of sites of 5-HT storage and metabolism by radioautography. *J. Physiol.*, **186,** 477–492.

GOLDSTEIN, A. (1965). *Biostatistics: An Introductory Text*, 2nd ed. New York: The Macmillan Co.

JONAKAIT, G.M., TAMIR, H., RAPPORT, M., & GERSHON, M.D. (1977). Detection of a soluble serotonin-binding protein in the mammalian myenteric plexus and other peripheral sites of serotonin storage. *J. Neurochem.*, **28,** 277–284.

LINEWEAVER, A. & BURK, D. (1934). The determination of enzyme dissociation constants. *J. Am. Chem. Soc.*, **56,** 6:

WONG, D.T., HORNG, J.S., BYMASTER, F.P., HAUSER, K.L. & MOLLOY, B.B. (1974). A selective inhibitor of serotonin uptake: Lilly 110140, 3-(b-trifluoromethylphenoxy)-N-methyl-3-phenylpropylamine. *Life Sci., Oxford*, **15,** 471–479.

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