Increase of pineal noradrenaline concentration in rats by desipramine but not fluoxetine: implications concerning the specificity of these uptake inhibitors

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Reuptake across the neuronal membrane appears to be the major mechanism by which neurotransmitter monoamines are inactivated after release into the synaptic cleft. This neuronal reuptake process is readily demonstrated in brain slices or in synaptosomes *in vitro*. Some inhibitors of amine uptake are remarkably selective in blocking the uptake of 5-hydroxytryptamine (5-HT) or noradrenaline. For example, desipramine is a selective inhibitor of noradrenaline uptake, whereas fluoxetine is a selective inhibitor of 5-HT uptake *in vitro* and *in vivo* (Fuller & Wong, 1977). A question that sometimes arises is whether the selectivity of an uptake inhibitor relates to the uptake pump or to the substrate. That is, does fluoxetine inhibit uptake of any amine by the pump on 5-HT neuronal membranes? Or, does fluoxetine inhibit 5-HT uptake, whether that uptake is by 5-HT neuronal membrane pumps or by other pumps, such as on catecholamine neurons? The latter question becomes important in interpreting certain pharmacological experiments. For instance, when 5-hydroxytryptophan is injected, some of it may be decarboxylated to 5-HT in cells other than 5-HT neurons. If it is decarboxylated in catecholamine neurons, the 5-HT so formed may influence their functioning either by displacing some catecholamine from storage granules or by being released as a false neurotransmitter. If an uptake inhibitor is specific for a neuronal pump, then enhancement by fluoxetine of some effect of injected 5-hydroxytryptophan can properly be interpreted as indicating that action is mediated by 5-HT neurons.

There are probably few instances in which an amine is taken up physiologically by a neuronal pump other than the one on the neuron that released that amine. One situation in which 5-HT is taken up by noradrenaline neurons under physiological conditions appears to be in the pineal gland (Jaim-Etcheverry & Zieher, 1968). Noradrenaline nerve fibres innervate the pineal gland, releasing noradrenaline and re-taking it up as a means of terminating its action on receptor sites on the pineal cells. The pineal cells synthesize large quantities of melatonin, with 5-HT as an intermediate. Because the concentrations of 5-HT in the pineal gland are large compared with those of noradrenaline, the uptake pumps on the noradrenaline nerve membrane 'accidentally' suck in substantial quantities of 5-HT along with the noradrenaline. This system— a noradrenaline pump taking up 5-HT— provides an uncommon opportunity to study the specificity of an uptake inhibitor. Which inhibitors block this uptake of 5-HT by a noradrenaline pump?

As we are not equipped to do the ultrastructural cytochemistry necessary to estimate 5-HT content within nerve terminals, our approach to this question has been based on the observation by Jaim-Etcheverry & Zieher (1971) that depletion of 5-HT from the noradrenaline nerve terminals increased the concentration of noradrenaline in the pineal gland. They reasoned that depleting 5-HT in the nerve terminals made additional granular storage sites available to noradrenaline. As more noradrenaline was retained in the storage granules, the extragranular cytoplasmic concentration of noradrenaline decreased, resulting in increased synthesis of noradrenaline due to release of feedback inhibition at the rate-limiting tyrosine hydroxylation step. Therefore we compared the effects of fluoxetine and desipramine on pineal noradrenaline concentration.

Male Wistar rats, about 150 g (Harlan Industries, Cumberland, Indiana) were given inhibitor (20 mg kg^{-1}) and decapitated up to 24 h later. The pineal glands were rapidly removed and frozen in a 1·5 ml plastic centrifuge tube containing 0·2 ml 0·1 M $HClO_4$ + 1 mM $NaHSO_3$ as an antioxidant. The pineal was homogenized on the following day by sonication for 5 s. The homo-

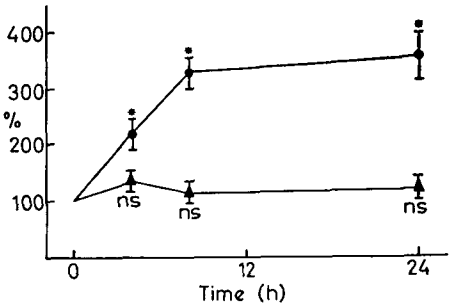

FIG. 1. Elevation of pineal noradrenaline concentration by desipramine (●) but not fluoxetine (▲). Desipramine and fluoxetine were injected intraperitoneally 20 mg kg^{-1}. Pineal noradrenaline concentration was calculated as ng mg^{-1} protein and is expressed as percentage of the mean value of control rats included in each experiment. Mean percentages ± standard errors for 5 rats per group are shown. Asterisks indicate data points that differed significantly from the corresponding control group ($P < 0.05$); 'ns' indicates no significant drug effect. Three control groups had pineal noradrenaline concentrations of 11·4 ± 1·4, 11·0 ± 1·0, and 9·6 ± 2·0 ng mg^{-1} protein.

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genate was kept in an ice bath before centrifugation at 12 000 *g* for 5 min. The supernatant fraction was carefully drawn off with a microlitre pipette and assayed for noradrenaline essentially as described by Keller, Arvin & others (1976). Briefly, the method involves adsorption of noradrenaline onto alumina followed by high pressure liquid chromatography with electrochemical detection of the eluted noradrenaline. The high sensitivity of this assay enabled us to measure the small amount of noradrenaline (1–2 ng) present in a single pineal. Data were more consistent when noradrenaline concentration was expressed in ng mg^{-1} protein, though conclusions about drug effects were the same when noradrenaline concentration was expressed as ng per pineal gland. Protein was assayed conveniently on the $HClO_4$ precipitate by dissolving the pellet in 0·5 M NaOH (0·5 ml), adding 0·5 ml 0·1 M tris buffer pH 7, and measuring absorbance at 280 and 260 nm. Protein content was calculated from the data of Warburg & Christian as described by Layne (1957).

Fig. 1 shows the concentration of pineal noradrenaline during a 24 h period after injection of the uptake inhibitors. Noradrenaline was significantly increased within 4 h after desipramine injection and remained so at the end of 24 h. These findings are in agreement with the report of Jaim-Etcheverry & Zieher (1971). Another uptake inhibitor, EXP 561* which inhibits uptake into noradrenaline and 5-HT neurons (Fuller & Wong, 1977), produced a similar increase in pineal noradrenaline concentration. Eight h after EXP 561 (10 mg kg^{-1}, i.p.), pineal noradrenaline concentration was increased from the control value of 11·2 ± 2 to 27·4 ± 4 ng mg^{-1} protein. In contrast, fluoxetine (20 mg kg^{-1}) did not lead to a significant change in noradrenaline concentration at any of the times measured. This dose of fluoxetine is twice that previously shown to inhibit uptake into brain 5-HT neurons for more than 24 h as measured by block of *p*-chloroamphetamine depletion of 5-HT (Fuller, Perry & Molloy, 1975).

In vitro studies with brain synaptosomes and *in vivo* studies evaluating uptake in brain and heart have established that desipramine is a highly selective inhibitor of noradrenaline and not 5-HT uptake, whereas fluoxetine is a highly selective inhibitor of 5-HT uptake (see Fuller & Wong, 1977). Our present results with the pineal gland indicate that desipramine is effective in blocking the uptake of 5-HT into the noradrenaline nerve terminals, whereas fluoxetine is not. These data are interpreted as confirming that the specificity of uptake inhibitors of this kind resides in the uptake pump rather than the substrate. This idea is mechanistically sound, since the interaction of the inhibitors is not thought to involve combination with the substrate molecule, but rather combination with the receptor macromolecule so as to compete with the substrate attachment.

* 4-Phenyl-bicyclo(2,2,2)octan-1-amine HCl monohydrate.

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The opiate anomalies — another possible explanation?

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The opium alkaloid morphine (I), with its complex structure and wide range of pharmacological properties, was the starting point of a vast research effort. As structure-activity data have accumulated through the years, interesting hypothetical models of the opiate receptor have been advanced.

The first (cf. II) was due to Beckett & Casy (1954) who argued that, unlike more flexible molecules, morphine with its rigid structure and defined absolute stereochemistry was surrendering a lot of information about the three-dimensional nature of the receptor at which it exerted its biological effect. This model was extended (cf. III) by Bentley, Cowan & Lewis (1971) to accommodate the very potent Diels-Alder adducts (e.g. IV) derived from thebaine, which were believed to have unearthed another point of receptor contact.

A unique aspect of the structure of morphine (cf. I) is an aromatic ring held rigidly axial to a piperidine ring and this is a key feature in the design of model II. However, biological results on a range of synthetic pairs of isomers, V (Casy & Coates, 1974; Clarke, Kullnig & Martini, 1975), VI (Smissman & Steinman, 1966) and VII (Kupferberg, Mikhail & Portoghese, 1968) in which R = Ph and R′ = CO_2Et (·O·COEt in VI) or vice versa, suggested rather surprisingly that the position of the phenyl group in space with respect to the nitrogen atom