Effects of noradrenaline pump blockers on its uptake by synaptosomes from several brain regions; additional evidence for dopamine terminals in the frontal cortex

Desipramine (Iversen, 1965), protriptyline (Carlsson & Waldeck, 1965) and Lu 3-010 (3,3-dimethyl-1-(3-methyl aminopropyl)-1-phenylphthalane) (Waldeck, 1968) are potent inhibitors of noradrenaline uptake by peripheral sympathetic nerves. The study of noradrenaline uptake by brain slices or synaptosomes is complicated by the existence of a highly active catecholamine transport system in dopaminergic neurons (Snyder & Coyle, 1969; Coyle & Snyder, 1969a) in addition to a less active catecholamine transport system in noradrenergic nerves. Therefore striatal synaptosomes have often been used as a pure dopamine uptake system while hypothalamus has been used as a source of noradrenaline synaptosomes (Horn, Coyle & Snyder, 1970). Desipramine and Lu 3-010 seem to be highly selective noradrenaline pump blockers with very weak inhibitory effects on the dopamine and 5-HT pumps Häggendal & Hamberger, 1967; Carlsson, Fuxe & others, 1969). Benztropine, on the other hand, is among the most potent known blockers of the dopamine pump (Coyle & Snyder, 1969b), but also blocks the noradrenaline pump with about equal potency (Farnebo, Fuxe & others, 1970).

Cerebral cortex has generally been regarded as being relatively devoid of dopamine terminals while having a moderate density of noradrenaline terminals (Andén, Dahlström & others, 1966; Ungerstedt, 1971). However, reports concerning the effects of desipramine, protriptyline and Lu 3-010 on the uptake of noradrenaline by cortical slices or synaptosomes are conflicting. For example, Carlsson & others (1969) found Lu 3-010 to be a weak inhibitor of noradrenaline accumulation in cortex slices, in contrast to its strong action on peripheral organs. On the other hand, Ross & Renyi (1967) found Lu 3-010 to be an extremely potent inhibitor of noradrenaline uptake by slices of mouse cerebral cortex.

Recent evidence suggesting the existence of a relatively high density of dopamine terminals in the more frontal parts of the cortex (Thierry, Stinus & others, 1973; Björklund, Lindvall & Moore, personal communication; Lidbrink & Jonsson, 1973) led us to investigate the effects of desipramine, protriptyline, Lu 3-010 and benztropine on the uptake of noradrenaline by synaptosomes prepared from several brain regions. In preliminary experiments using synaptosomes from whole cortex, desipramine and Lu 3-010 exhibited partial plateau inhibition (10–40%) in concentrations ranging from 0·001 to 0·1 μM.

In subsequent experiments 7 pump blockers were tested for effects on uptake of noradrenaline and 5-HT by synaptosomes prepared from whole fore-brain (anterior to colliculus superior), frontal cortex, occipital cortex and hippocampus. Synaptosomes were prepared and incubated essentially according to the method of Kuhar, Roth & Aghajanian (1972) with the following modifications: all brain regions were homogenized in 5 volumes of 0·32 M sucrose except for whole forebrain (10 volumes) and striatum (30 or 60 volumes). Ice cold Krebs-Ringer phosphate was saturated with pure O_2 (without CO_2). Synaptosomes were incubated for 20 min at 37° in the modified Krebs-Ringer containing [^{3}H]noradrenaline (0·01 μM) and [^{14}C]-5-HT (0·045 μM), returned to an ice bath to stop uptake and the synaptosomes collected on Millipore filters (HAWOP 2500) with 0·45 μm pore diameter. After washing twice with 0·9% NaCl, the filters were placed in counting vials containing 10 ml Instagel. Tritium and Carbon-14 were counted simultaneously in a Packard Tricarb scintillation spectrometer. The blank value consisted of synaptosomes incubated as described

Table 1. *Inhibition of noradrenaline (NA) and 5-HT uptake into synaptosomes prepared from frontal cortex, occipital cortex, hippocampus, and whole fore-brain.*

	pIC50							
	Occipital cortex		Frontal cortex		Hippocampus		Whole fore-brain	
Substance	NA	5-HT	NA	5-HT	NA	5-HT	NA	5-HT
Desipramine ..	>9·	5·6	5·0	5·6	>9·	5·5	5·0	5·7
Protriptyline ..	8·3	5·7	5·5	5·6	8·7	5·9	5·5	5·7
Lu 3-010 ..	>9·	5·5	4·5	5·3	>9·	5·7	4·4	5·5
Imipramine ..	7·5	6·5	5·6	6·5	8·2	6·6	5·0	6·3
Chlorimipramine	7·5	8·2	5·6	8·1	8·2	8·3	5·5	7·4
Amitriptyline ..	7·3	6·4	5·6	6·4	7·5	6·3	5·5	6·3
Benztropine ..	6·4	4·5	6·7	4·5	6·6	4·5	6·6	4·4

above with the addition of 0·1 mM each of benztropine and chlorimipramine, and was usually about 5 to 10% of the control values. Test substances were serially diluted by a factor of 10 to give final concentrations ranging from 0·001 to 100 μM. The molar concentrations producing 50% inhibition of amine uptake were estimated by simple graphic interpolation, and the results expressed as pIC50 (the negative logarithm of the IC50).

In Table 1 it can be seen that desipramine, Lu 3-010 and protriptyline inhibit uptake of noradrenaline by synaptosomes prepared from frontal cortex or whole forebrain at concentrations 1000 to 10 000 times greater than those required to inhibit noradrenaline uptake into synaptosomes from occipital cortex or hippocampus.

Benztropine, on the other hand, was about equally potent in inhibiting noradrenaline uptake in all brain regions tested. This was in agreement with the results of Farnebo & others (1970) who showed that benztropine is about equally potent in inhibiting dopamine and noradrenaline uptake into neostriatal slices and isolated irides, respectively. Imipramine, chlorimipramine and amitriptyline were also much stronger inhibitors of noradrenaline uptake in occipital cortex and hippocampus synaptosomes than in synaptosomes from frontal cortex or whole forebrain, but with these 3 substances the potency differed only by a factor of 100 to 1000.

These results suggest that noradrenaline is taken up mainly by dopamine terminals in frontal cortex and whole forebrain. This might be expected in the case of whole forebrain since it contains the striatum, a region with a very high catecholamine uptake capacity compared with other brain regions (Snyder & Coyle, 1969). However, the failure of low concentrations of the potent noradrenaline pump blockers to inhibit noradrenaline uptake by frontal cortex synaptosomes was unexpected. The finding that benztropine inhibits this uptake with about the same potency as noradrenaline uptake into whole forebrain synaptosomes, taken together with the recent histochemical results of Björklund & others (personal communication) and of Lidbrink & Jonsson (1973), also strongly suggests that frontal cortex contains a surprisingly high density of dopamine terminals.

On the other hand, catecholamines seem to be accumulated almost entirely by noradrenaline synaptosomes prepared from hippocampus and occipital cortex since this accumulation can be inhibited 50% by desipramine, protriptyline and Lu 3-010 at concentrations of about 0·001 μM, and more than 90% at a concentration of 0·1 μM which is without major effect on the uptake of noradrenaline by synaptosomes prepared from frontal cortex or whole forebrain. Preliminary experiments done in this laboratory indicate that parietal cortex also contains a relatively high density of nerve terminals possessing properties of dopamine terminals, while catecholamine uptake by

synaptosomes prepared from pons-medulla, colliculi-tegmentum and cerebellum regions has properties of a relatively pure noradrenaline uptake system.

It is interesting that several of the brain regions found by us to exhibit desipramine and Lu 3-010 sensitive noradrenaline uptake (hippocampus and pons-medulla) have been shown by an autoradiographic study to contain terminals whose perikarya are located in the locus coureleus (LC) (Segal, Pickel & Bloom, 1973). These workers also found LC projection to the neocortex but, except for the occipital cortex, catecholamine uptake into noradrenaline terminals in cortex seems to be masked by a much more active uptake into dopamine terminals.

Lu 3-010 at 0·01–0·1 μM inhibited noradrenaline uptake into hypothalamus synaptosomes less than uptake into synaptosomes from pons medulla, colliculi-tegmentum, occipital cortex and hippocampus which in all cases was more than 90% at 0·1 μM. The results with hypothalamus may indicate the presence of dopamine terminals in this region as also suggested by Cuello, Horn & others (1973) who found significant desipramine resistant dopamine uptake in the median eminence and arcuate nucleus regions of the hypothalamus.

Synaptosomal uptake of 5-HT has similar properties in all regions of the brain examined. Of the 7 substances tested chlorimipramine was the most potent 5-HT uptake inhibitor while benztropine was the weakest in all regions (chlorimipramine > imipramine = amitriptyline > protriptyline = desipramine = Lu 3-010 > benztropine in all 4 preparations). These results indicate clearly that 5-HT, noradrenaline and dopamine are each taken up by specific mechanisms, in agreement with the findings of others (e.g. Kuhar & others, 1972).

Preliminary experiments also suggest that catecholamine uptake by synaptosomes from occipital and frontal cortex of the mouse has the properties of noradrenaline and dopamine pumps, respectively, as defined here.

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Antagonistic effects of apomorphine and haloperidol on rat striatal synaptosomal tyrosine hydroxylase

Haloperidol and several other antipsychotic neuroleptics have been shown to accelerate the turnover of dopamine in brain (Carlsson & Lindqvist, 1963; Andén, Corrodi & Fuxe, 1972; Andén, Roos & Werdinius, 1964; Cheramy, Besson & Glowinski, 1970) as well as to accelerate the rate of firing of dopamine neurons (Bunney, Walters & others, 1973). There is now good evidence that this effect of neuroleptics is a consequence of both pre- and post-synaptic (Kehr, Carlsson & others, 1972; Bunney & Aghajanian, 1973) dopamine receptor blockade, although the exact mechanisms involved remain to be elucidated. Apomorphine, a substance which appears to directly stimulate dopamine receptors, exerts effects on dopamine turnover opposite to those produced by neuroleptics: it retards the turnover of dopamine, which has also been measured in several ways (Roos, 1969; Nybäck, Schubert & Sedvall, 1970; Goldstein, Freedman & Backstrom, 1970), inhibits the activity of dopamine neurons (Bunney, Aghajanian & Roth, 1973) and antagonizes the effects of neuroleptics (Andén, Rubenson & others, 1967; Lahti, McAllister & Wozniak, 1972; Kehr & others, 1972).

The interruption of impulse flow in dopamine neurons, either by axotomy or by the administration of γ-hydroxybutyric acid, causes a 60 to 100% increase in striatal dopamine (Walters, Roth & Aghajanian, 1973) an effect that can be blocked by apomorphine (Andén, Magnusson & Stock, 1973). Furthermore, the *in vivo* inhibition of tyrosine hydroxylase by apomorphine in denervated forebrain was shown to be partially reversed by the simultaneous administration of haloperidol (Kehr & others, 1972). These findings strongly suggest that dopamine neurons are, at least partly, autoregulated; released or extraneuronal dopamine seems to act on presynaptic, inhibitory dopamine receptors. Stimulation of the inhibitory dopamine receptors on dopamine neurons, either by dopamine or apomorphine, may inhibit both nerve activity and dopamine synthesis.

Striatal synaptosomes have relatively high tyrosine hydroxylase activity (McGeer, Bagchi & McGeer, 1965; Cicero, Sharpe & others, 1972), which is not dependent on added cofactors in contrast to non-synaptosomal tyrosine hydroxylase (Karobath, 1971). Since synaptosomes may function as a pure *in vitro* pre-synaptic system, with little or no influence by post-synaptic events, we decided to test the effects of apomorphine and haloperidol on tyrosine hydroxylase activity in striatal synaptosomes.

Tyrosine hydroxylase activity was measured both as $^3H\text{-}H_2O$ formed according to Nagatsu, Levitt & Udenfriend (1964) and accumulated [^{3}H]catecholamines according to the slightly modified procedure of Renzini, Brunori & Valori (1970) from L[^{3}H]3,5-tyrosine (70 Ci mmol^{-1}), which was purified on a Dowex-50 column from which it was eluted with N ammonium hydroxide. The eluate was stored at 4° for up to 14 days. Just before use a suitable volume of the tyrosine eluate was evaporated to dryness at room temperature (20°) under a stream of nitrogen, and taken up in oxygen-saturated Krebs-Ringer phosphate containing glucose, ascorbate and EDTA as described by Kuhar, Roth & Aghajanian (1972) to give about 2 million counts min^{-1} per assay.