L-Dopa induced changes in central monoamine neurons after peripheral decarboxylase inhibition*

The functional and the biochemical effects of L-3,4-dihydroxyphenylalanine (L-dopa) treatment on central monoamine neurons after extracerebral dopa-decarboxylase inhibition have recently been published (see Butcher & Engel, 1969). Small doses of Ro 4–4602, *N*-(DL-seryl)-*N*-(2,3,4-trihydroxybenzyl)hydrazine, an inhibitor of the decarboxylase of aromatic amino-acids, selectively inhibit extracerebral dopa-decarboxylase. At these doses the enzyme in brain is relatively unaffected (Bartholini, Bates & others, 1967). Formation of dopamine in the brain after systemic injection of L-dopa in rats is enhanced by pretreatment with Ro 4–4602 (Bartholini & others, 1967; Butcher & Engel, 1969). At the same time there is a reduction of central 5-hydroxytryptamine (5-HT) concentrations (Bartholini, Da Prada & Pletscher, 1968; Butcher & Engel, 1969) and a slight reduction of noradrenaline (Butcher & Engel, 1969). Furthermore, extracerebral dopa-decarboxylase inhibition by Ro 4–4602 induces an increased formation of catecholamines from L-dopa in the neuropil and a reduced formation of catecholamines in the walls of the capillary cells (Constantinidis, Bartholini & others, 1968). However, no detailed histochemical analyses of the localization and the distribution of the central monoamines after combined Ro 4–4602–L-dopa treatment have been made. We now give a detailed description of L-dopa-induced changes in central monoamine neurons after extra-cerebral decarboxylase inhibition by small doses of Ro 4–4602. We have used a fluorescence method (Falck, Hillarp & others, 1962; Hillarp, Fuxe & Dahlström, 1965).

Adult male Sprague-Dawley rats, 160–180 g, were decapitated after chloroform anaesthesia and all parts of the brain were taken for histochemical fluorescence analyses of catecholamines and 5-HT. In each experiment the brains of 8–10 rats were analysed using coded slides. Ro 4–4602 (50 mg/kg, i.p.) was given 30 min before the L-dopa injection (50; 100; 200 mg/kg, i.p.). The rats were killed 75 min after the L-dopa injection.

Central dopamine neurons. There was a dose-dependent increase in the fluorescence intensity of the catecholamine cell body groups of the mesencephalon (Dahlström & Fuxe, 1964) which are all dopamine cell bodies (Corrodi, Fuxe & others, 1970). A very strong fluorescence intensity was found after 100–200 mg/kg of L-dopa in combination with Ro 4–4602 in the dopamine cell bodies of the zona compacta (lateral part), the pars lateralis and the zona reticulata of the substantia nigra, and also the mesencephalic reticular formation and the arcuate nucleus. The entire pericaryon became fluorescent together with its processes. The nuclei could no longer be observed. After treatment with L-dopa alone in the same doses and at the same time-interval, only a medium to strong fluorescence intensity was observed but this is above normal.

A dose-dependent increase in fluorescence intensity was also observed in the nigro-neostriatal dopamine fibres and in the dopamine nerve terminal systems. In the latero-dorsal part of the neostriatum, those nerve terminal systems show more marked increases than those in the medio-ventral part and those in the limbic fore-brain. In view of the fact that the dopamine cell bodies in the lateral part of the substantia nigra and in the mesencephalic reticular formation showed the most marked increase after Ro 4–4602 - L-dopa treatment, it may be that these dopamine cell bodies mainly innervate the lateral-dorsal part of the neostriatum (this corresponds mainly to putamen). The existence of a lateral nigroneostriatal dopamine pathway has also been suggested by Poirier, McGeer & others (1969). No increase in fluorescence intensity was observed in the dopamine nerve terminals of the median

eminence. This could be because this area is localized outside the blood-brain barrier. Thus, Ro 4–4602 can reach this area and block the decarboxylation of L-dopa to dopamine.

Central noradrenaline neurons. No increases in fluorescence intensity were observed in the noradrenaline cell bodies of the medulla oblongata and the pons (see Andén, Dahlström & others, 1966), or in the noradrenaline nerve terminal systems. At the highest dose of L-dopa after Ro 4-4602 pretreatment, these terminals appeared indistinct because of the increased background fluorescence in the neuropil, especially in areas having higher densities of the terminals. These changes were probably partly the result of an outflow of newly formed catecholamines, mainly dopamine, (from dopa) over the noradrenaline nerve cell membrane into the extraneuronal space.

Central 5-HT neurons. The 5-HT cell bodies became distinctly greenish when seen in the fluorescence microscope. This fluorescence was mainly localized to a perinuclear ring and was of weak to medium intensity. The 5-HT nerve terminals and fibre bundles exhibited a very weak to weak greenish fluorescence.

Extraneuronal tissue. There was a dose-dependent increase in the diffuse green fluorescence of the neuropil in agreement with Constantinidis & others (1968). Furthermore, the fluorescence in the cells of capillary walls was much reduced compared with that from cells of rats treated with dopa alone. In the highest dose, however, a distinct weak green fluorescence was still seen in the capillary cells in most parts of the brain in spite of Ro 4–4602 pretreatment.

When L-dopa alone is given systemically to rats it is probably mainly decarboxylated extracerebrally because the dopa-decarboxylase activity is much higher in peripheral organs than in the brain (Blaschko & Chruściel, 1960) and also, it has been shown that the dopa-decarboxylase localized in the cells of the brain capillaries constitutes an enzymatic barrier for dopa between blood and brain. Thus, when the extra cerebral dopa-decarboxylase is inhibited by Ro 4–4602 and the blood-brain barrier for dopa is broken there is, as we observed, a high uptake and decarboxylation of dopa in the central monoamine neurons. The result is a marked accumulation of dopamine in these neurons especially in some dopamine nerve cells. At the time-interval studied, these accumulations of dopamine must partly be located extra-granularly since a strong fluorescence also was observed in the fibre bundles which contain only a few dopamine granules. A high reserve capacity of these granules to store dopamine may also contribute to the marked accumulation of dopamine observed.

The results of the present paper have also contributed to the mapping out of central dopamine neurons. Thus, the fact that certain dopamine cell bodies and terminals in the mesencephalon and the lateral neostriatum respectively show very marked accumulation of dopamine after Ro 4–4602 - L-dopa treatment, suggests that these structures belong to the same dopamine nerve cells forming a lateral nigro-neostriatal pathway from the lateral part of the substantia nigra and the mesencephalic reticular formation.

The central noradrenaline neurons do not show any increase or decrease in their fluorescence intensity. In view of the biochemical results (Butcher & Engel, 1969) demonstrating a slight decrease in brain noradrenaline levels after Ro 4–4602 – L-dopa treatment, the histochemical results suggest that the dopamine formed intraneuronally from exogenous dopa in the central noradrenaline neurons can displace noradrenaline from the amine granules in those neurons. These results also underline the view that the storage capacity for dopamine and noradrenaline in the amine granules in the noradrenaline neurons may be more or less saturated already in the physiological state. The results of Corrodi & Fuxe (1967) support this; they found nialamide not

to cause any further increase in the accumulation of noradrenaline in the noradrenaline neurons after dopa injection in rats depleted of their noradrenaline stores by a tyrosine hydroxylase inhibitor. Another operating mechanism may be that the β-hydroxylation of dopamine to noradrenaline is the rate-limiting step in its synthesis in the central noradrenaline neurons, but this has not been proved. The possibility that there is a relatively poor uptake of dopa into the noradrenaline neurons compared with the dopamine neurons must also be considered.

The accumulation of dopamine in the 5-HT neurons we observed suggests that the depletion of 5-HT stores after Ro 4–4602 - L-dopa treatment may be due to a displacement mechanism. The results of Bartholini, Da Prada & Pletscher (1968) support this; they found that the concentrations of 5-hydroxyindoleacetic acid increased after treatment with Ro 4–4602 and L-dopa. A decreased 5-HT synthesis induced by L-dopa may, however, contribute to the depletion of the central 5-HT levels, since the injected L-dopa may compete with the normally available tryptophan and 5-hydroxytryptophan for the entry into the brain and probably also over the 5-HT nerve cell membrane (Bartholini, Da Prada & Pletscher, 1968; unpublished data).

The present results differ from those obtained with L-dopa without extracerebral dopa decarboxylase inhibition where there is a relatively selective uptake and decarboxylation of dopa in central catecholamine neurons. This can be observed when L-dopa is injected to rats treated with a monoamine-oxidase inhibitor or a tyrosine hydroxylase inhibitor (Fuxe, 1965; Corrodi, Fuxe & Hökfelt, 1966; Corrodi & Fuxe, 1967). The present data indicate that this relative degree of specificity is abolished when loading the brain with high amounts of dopa by way of extracerebral decarboxylase inhibition.

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Reversal by trypsin of the action of a 2-haloalkylamine

N-(2-bromo-ethyl)-*N*-ethyl-1-naphthalenemethylamine hydrobromide (SY28) is a potent member of a group of compounds, the 2-haloalkylamines, which are notable for producing an insurmountable antagonism to the motor action of noradrenaline on smooth muscle (Nickerson & Goodman, 1946; Graham, 1962). This blockade has been attributed to alkylation of the specific α-receptor (Harvey & Nickerson, 1954) and the nature of the bonding has been the subject of long research and much speculation (Belleau, 1958, 1959). In 1966 Graham & Katib described how addition of trypsin to a bath containing an isolated vas deferens of the guinea-pig, in which insurmountable antagonism to added noradrenaline had been produced by prior exposure of the tissue to a fully blocking dose of one of three 2-haloalkylamine compounds, reversed the blocking action. An attempt to repeat this work on rabbit vas has been reported (Moran, May & others, 1967) to have given equivocal results. Accordingly, the experiment has been repeated. Vasa from 400 g guinea-pigs were suspended in 10 ml of Hukovic solution at 37°, gassed with 5% carbon dioxide in oxygen, six together at a time, and stimulated 5 times with addition of noradrenaline 10^{-8} g/ml. ^{14}C-SY28 was then added to the bath for 20 min in a concentration of $1{\cdot}34 \times M^{-6}$ containing $3{\cdot}35 \times 10^{-4}$ mCi in the 10 ml. Insurmountable antagonism to noradrenaline was then demonstrated, the tissue washed 12 times at 3 min intervals

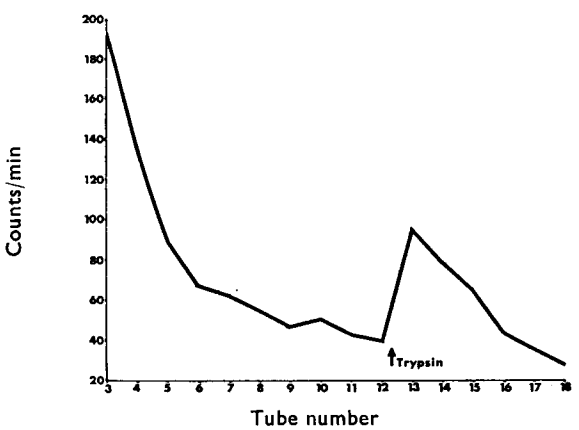

FIG. 1. The scintillation count in counts/min in the fluid in which was suspended guinea-pig vas deferens exposed to ^{14}C-SY28 $1{\cdot}34 M^{-6}$ in 10 ml for 20 min. Each tube no. refers to one 3 min cycle and a change of bath fluid. Trypsin $2{\cdot}5\ 10^{-3}$ BAEE units/ml was added for 2 min at arrow.