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DL-5-Hydroxytryptophan-induced changes in central monoamine neurons after peripheral decarboxylase inhibition*

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The histochemical effects of 500 and 1000 mg/kg of DL-5-hydroxytryptophan (5-HTP), both alone and in combination with a peripheral decarboxylase inhibitor (seryl-trihydroxy benzyl hydrazine; Ro 4-4602) have been examined on central monoamine neurons of rats by the Falck-Hillarp fluorescence technique that demonstrates monoamines and their precursors. 5-HTP alone or together with Ro 4-4602 caused only weak intraneuronal accumulation of 5-HT in the central 5-HT neurons, in spite of an increased entry of 5-HTP into the brain after Ro 4-4602 treatment, as shown by an increase in the specific neuropil fluorescence and a reduction of 5-HT accumulation in the cells of the capillary walls. Ro 4-4602 markedly potentiated the effects of 5-HTP on the central dopamine neurons, many of which became clearly yellow fluorescent. The mechanism of dopamine depletion by 5-HTP is probably therefore mainly one of displacement. The effects on the noradrenaline neurons were also potentiated by Ro 4-4602 pretreatment, the neurons exhibiting a yellow-green fluorescence. This depletion may therefore also be mainly be due to amine displacement. It is concluded that the ability of the 5-HT neurons to take up and accumulate 5-HT in the presence of 5-HTP is relatively low in spite of large amounts of 5-HTP present in the brain neuropil after extracerebral decarboxylase inhibition.

Recently a detailed histochemical analysis of the effects of L-3,4-dihydroxyphenylalanine (L-dopa) on central monoamine neurons after extracerebral decarboxylase inhibition was presented (Butcher, Engel & Fuxe, 1970). It was reported that in the presence of the decarboxylase inhibitor Ro 4-4602 [N^1-(DL-seryl)-N^2-(2,3,4-trihydroxybenzyl)hydrazine, (Bartholini & Pletscher, 1968)], the intraperitoneal injection of L-dopa to rats resulted in a marked accumulation of dopamine in central dopamine neurons. The intraneuronal dopamine concentrations in dopamine cell bodies, nonterminal axons, and nerve terminals were much increased compared with those obtained when L-dopa was used alone. It was also observed that the 5-hydroxytryptamine (5-HT) neurons contained catecholamines that had probably displaced existing 5-HT stores. This poses the question whether a similar picture might emerge after administration of 5-hydroxytryptophan (5-HTP), the precursor of 5-HT. Using a histochemical fluorescence analysis of monoamines (Falck, Hillarp & others, 1962; Hillarp, Fuxe & other, 1965; Corrodi & Jonsson, 1967), we have assessed the effects of DL-5-HTP on central monoamine neurons after peripheral decarboxylase inhibition by Ro4–4602.

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MATERIAL AND METHODS

Male Sprague-Dawley rats (150–180 g) were injected intraperitoneally either with DL-5-HTP alone (dissolved in 0·9% saline with warming in doses either of 500 or 1000 mg/kg), six rats being in each dosage group; or with 5-HTP preceded by Ro 4-4602 (50 mg/kg in saline) 30 min before the 5-HTP administration; eight rats being in each dosage group. All animals were killed 75 min after precursor administration.

Normal untreated rats were included in each experiment, as were other control animals injected with saline alone in volumes comparable to those used in the experimental groups. Since Butcher & Engel (1969) and Butcher & others (1970) have shown that R o4-4602 (50 mg/kg) injected alone does not affect central monoamine levels, a separate control with this drug treatment was not included.

Immediately after death, brain and spinal cord were taken for histochemical fluorescence analyses of catecholamines and 5-HT (Dahlström & Fuxe, 1965; Fuxe & Jonsson, 1967). Serial transverse sections were made of all regions in each animal. Semi-quantitative estimations of fluorescence intensity were made on coded slides. It is known that a change in fluorescence intensity reflects a change in amine concentration (Olson, Hamberger & others, 1968; Jonsson, 1969).

RESULTS

DL-5-*HTP alone*

5-HT neurons. A slight increase in the fluorescence intensity of the 5-HT cell bodies and nerve terminals in the entire central nervous system, was observed but only at the 1000 mg/kg dose.

Dopamine neurons. The dopamine nerve terminals in the median eminence, which lies outside the blood brain barrier, had a strong yellow fluorescence, probably due to the accumulation of 5-HT and subsequent displacement of dopamine stores. However, the dopamine nerve terminals of the limbic forebrain and in the neostriatum showed no alterations in fluorescence intensity. After the high dose of 5-HTP, however, the fluorescence in these latter two areas changed from green to yellow-green, suggesting an increase in indoleamine. The dopamine cell bodies in the hypothalamus and the mesencephalon showed a small decrease in fluorescence intensity and a shift in colour towards yellow-green.

Noradrenaline neurons. After 1000 mg/kg of 5-HTP there was a clear decrease in the fluorescence intensity of the noradrenaline nerve terminals. In most areas of the brain the fluorescence was green to yellow-green. No changes in fluorescence intensity and colour were observed in the cell bodies.

Cells of the capillary walls (pericytes and endothelial cells). After 500 mg/kg of 5-HTP the pericytes and endothelial cells showed a strong yellow fluorescence which was increased with the 1000 mg/kg dose.

Extraneuronal tissue. A small increase, compared with untreated controls, in fluorescence intensity was observed in the neuropil. The fluorescence had a yellowish colour.

5-*HTP in combination with Ro* 4-4602

5-HT neurons. A slight increase in fluorescence intensity was observed in the 5-HT cell bodies and nerve terminals. But whether the increase was greater than that obtained with 5-HTP alone could not be assessed.

Dopamine neurons. In contrast to the results obtained with 5-HTP alone, the yellow fluorescence in the dopamine nerve terminals in the median eminence was no longer observed, probably because the decarboxylase inhibitor could effectively reach this area, which lies outside the blood brain barrier. Therefore, the decarboxylation of 5-HTP could not occur to any great extent. Instead a diffuse yellowish fluorescence was seen throughout the median eminence probably due to the presence in the brain tissue of 5-HTP which itself is converted into a fluorescent β-carboline by the histochemical technique (see Corrodi & Jonsson, 1967). The dopamine nerve terminals in the neostriatum and limbic forebrain showed a shift in fluorescence from green to yellow-green. In the nucleus amygdaloideus centralis and in the dorsolateral part of the nucleus interstitialis striae terminalis, the dopamine nerve terminals were clearly yellow. These effects were obtained with both doses of 5-HTP, a dose-dependent relation being observed.

The dopamine cell bodies of the arcuate nucleus (group A 12 according to Dahlström & Fuxe, 1964), of the substantia nigra (group A 9), and of the ventrolateral part of the mesencephalic reticular formation (group A 8) developed a moderate to strong yellow fluorescence. Normally they exhibit a weak to moderate green intensity. The yellow fluorescence was weaker in the medial part of the substantia nigra although it was more intense in all cell bodies than that formed in the 5-HT cell bodies. The dopamine cell bodies of group A 10 displayed practically no yellow fluorescence but did have a yellow-green colour, of normal intensity. The dopamine cell bodies exhibiting the greatest accumulation of 5-HT were those showing the strongest accumulation of dopamine after dopa treatment (Butcher, Engel & others, 1970). The yellow fluorescence in the dopamine cell bodies was primarily localized in a perinuclear ring as was the endogenous green fluorescence.

Noradrenaline neurons. In contrast to the results with 5-HTP alone, a decrease in fluorescence intensity of the noradrenaline nerve terminals was seen with both less and high doses of 5-HTP. The colour of the fluorescence was yellow-green. The cell bodies were not substantially affected; if anything, a small decrease in intensity was noted.

Cells of the capillary walls. In contrast to the effect after 5-HTP alone, virtually no yellow fluorescence was evident in the pericytes and endothelial cells, even with the high dose of 5-HTP.

Extraneuronal tissue. A strong yellowish fluorescence was observed throughout the neuropil, possibly due to the presence in the brain tissue of 5-HTP itself.

DISCUSSION

Previously, a relative specificity for the 5-HT neurons in the uptake and decarboxylation of 5-HTP, in doses of 20–100 mg/kg, in combination with tryptophan hydroxylase inhibition or monoamine oxidase inhibition has been observed (Fuxe, 1965; Corrodi, Fuxe & Hökfelt, 1967). The presence of 5-HT derived from 5-HTP could be detected only in the 5-HT neurons. The present study has demonstrated that with 5-HTP in doses of 500 or 1000 mg/kg, especially in combination with a peripheral decarboxylase inhibitor, this relative specificity is lost, and changes in the overall fluorescence pattern are also seen in the central dopamine and noradrenaline neurons.

Brain levels of 5-HT are increased approximately 5 times normal after injection of 500 mg/kg of 5-HTP and about 8 times normal after 1000 mg/kg of 5-HTP (Butcher,

Engel & Fuxe: unpublished observations). These same ratios exist regardless of whether 5-HTP has been injected alone or in combination with an extracerebral decarboxylase inhibitor (Henning & Rubenson 1971; Butcher, Engel & Fuxe, unpublished data). Coupling these findings with the results obtained in the present experiments would suggest that the increase in brain 5-HT concentration after 5-HTP alone or in conjunction with an extracerebral decarboxylase inhibitor is mainly attributable to increased amounts of extraneuronal 5-HT, since there were but weak increases in intraneuronal indoleamine fluorescence. Thus, the 5-HT neurons would appear to react to 5-HTP treatment in a manner similar to the response of noradrenaline neurons after an injection of L-dopa (cf., Butcher & Engel, 1969; Butcher, Engel & others, 1970). That is, no increases in brain noradrenaline concentrations were observed in the noradrenaline neurons after combined Ro 4-4602 and L-dopa treatment. The fact that little accumulation of intraneuronal 5-HT was observed, even though high amounts of 5-HT are present as assessed biochemically, may be due to several factors: (1) formation of 5-HT in places other than the 5-HT neurons, e.g. in the dopamine neurons, (2) low uptake of 5-HTP into the 5-HT neurons, and (3) leakage of 5-HT from the neurons into the extraneuronal space. No obvious indication of accumulation of 5-HT around the 5-HT and dopamine neurons was seen however. Finally, the possibility cannot be excluded that less 5-HTP is decarboxylated in the 5-HT neurons than in the dopamine neurons.

The effects of 5-HTP on dopamine neurons were clearly potentiated by Ro 4-4602 pretreatment. In fact some of the dopamine cell bodies had a distinctly yellow fluorescence, with an intensity even higher than that found in the 5-HT neurons. In view of this, it is possible that the depletion of dopamine might be attributable to displacement of the dopamine stores by the 5-HT formed from 5-HTP. The findings that 5-HTP in combination with an extracerebral decarboxylase inhibitor results in a decrease in central dopamine concentrations lends further support to this contention (Henning & Rubenson, 1971; Butcher, Engel & Fuxe: unpublished observations). This mechanism would not be at variance with a similar hypothesis advanced to explain the depletion of central 5-HT administration of L-dopa in combination with Ro 4-4602 (Bartholini, DaPrada & others, 1968; Butcher & Engel, 1969).

The reason for the marked accumulation of 5-HT in the dopamine nerve cells may be due to the high capacity of these neurons to take up or to decarboxylate amine precursors, or both. With a high intraneuronal concentration of 5-HTP in dopamine neurons we would expect the 5-HT precursor to compete with normally available dopa for the decarboxylase enzyme, since the same enzyme is thought to catalyse the decarboxylation of both 5-HTP and dopa (Rosengren, 1960). This mechanism would also result in a net decrease of dopamine accompanied by an increase in 5-HT. Furthermore, the injected 5-HTP may compete with normally available L-dopa for entry into the brain.

With both doses of 5-HTP the depletion of noradrenaline by indoleamines is potentiated by Ro 4-4602 pretreatment. After high doses of 5-HTP, noradrenaline concentrations in mouse heart and brain (Andén, 1964) and in rat brain (Butcher, Engel & Fuxe. unpublished data) are reduced. The effect of 5-HTP on the noradrenaline neurons may be attributable to displacement of endogenous noradrenaline stores by the 5-HT formed.

When 5-HTP is given systemically to rats it is probably mainly decarboxylated extracerebrally since the decarboxylase activity is much higher in peripheral organs

like the kidney and the liver than in the brain (Hagen & Cohen, 1966), and, as suggested by the present experiments, the decarboxylase in the cells of the capillary walls may constitute a barrier against the entry of 5-HTP into the brain neuropil. However, by inhibiting the decarboxylase in the peripheral organs and in the cells of the capillary walls, using Ro 4-4602, the entry of 5-HTP into the brain tissue is facilitated, as shown by the marked increase in neuropil fluorescence. In spite of the fact that little intraneuronal 5-HT accumulation was observed, sufficient 5-HT must have been formed to reach the receptor sites since, as assessed by gross observation, the administration of 5-HTP alone, or in combination with Ro 4-4602, results in a syndrome characterized by immobilization, tremors in the head and the forelimbs, extension and abduction of the hindlimbs. Furthermore, the extensor hindlimb reflex activity of the spinal rat, which is dependent on the integrity of 5-HT receptor stimulation (Andén, 1968), is increased by combined Ro 4-4602–5-HTP treatment, but not appreciably by 5-HTP alone (Fuxe, unpublished observations). Finally, the present results illustrate the difficulties attendant on the correlation of histochemical, and particularly biochemical determinations of changes in 5-HT concentrations after 5-HTP with corresponding changes in function.

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