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Enhancement of 5-hydroxytryptamine synthesis in brain by monoamine-depleting drugs

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The turnover of monoamines such as dopamine in the brain is regulated by feedback mechanisms. Drugs which activate pre- and/or postsynaptic dopamine receptors (e.g. apomorphine) decrease the cerebral dopamine turnover, whereas compounds which inhibit the dopamine receptors or reduce the number of chemical stimuli reaching them (e.g. neuroleptics, monoamine depletors like reserpine) enhance the turnover of dopamine (Andén, Roos & Werdinius, 1964; Carlsson & Lindqvist, 1963; Corrodi, Fuxe & Hökfelt, 1967; Clement-Cormier, Kebabian & others, 1974; Carlsson, 1975). For the estimation of the rate of synthesis of dopamine or 5-hydroxytryptamine (5-HT), inhibitors of cerebral decarboxylase such as NSD 1015 (3-hydroxybenzylhydrazine HCl) and benserazid (1-DL-seryl-2(2,3,4-trihydroxybenzyl)hydrazine HCl) have been used (Carlsson, Davis & others, 1972). As a result of decarboxylase inhibition, the concentration of the endogenous precursors of these amines, i.e. 3,4-dihydroxyphenylalanine (dopa) and 5-hydroxytryptophan (5-HTP) respectively, increases and in situations where dopamine or 5-HT synthesis is accelerated, the increase in precursors is enhanced. In fact, reserpine (as do neuroleptic drugs) causes an enhancement of the NSD 1015-induced rise of endogenous cerebral dopa (Carlsson, 1975) together with an increase of homovanillic acid (Andén & others, 1964), a major metabolite of dopamine. These long-lasting (over 24 h) changes are thought to be due to an acceleration of dopamine turnover. The action of reserpine on 5-hydroxytryptaminergic neurons seems to differ from that on dopaminergic neurons. Thus, while the drug induces a long-lasting increase of 5-hydroxyindoleacetic acid (5-HIAA) (Roos, Andén & Werdinius, 1964; Tozer, Neff & Brodie, 1966), the major metabolite of 5-HT in the brain, no marked enhancement of the rise of 5-HTP induced by NSD 1015 has been observed 7–8 and 5 h after administration of reserpine to mice and rats respectively (Carlsson & Lindqvist, 1972; Modigh, 1974).

The present paper deals with the effect of reserpine and of a benzoquinolizine derivative with a short-lasting, reserpine-like action (2-hydroxy-2-ethyl-3-isobutyl-9,10-dimethoxy-1,2,3,4,6,7-hexahydro-11bH-benzo[a]quinolizine HCl, Ro 4-1284; Pletscher & Da Prada, 1966) on 5-HT synthesis in rat brain.

Male albino rats (Füllinsdorf breed of Wistar origin, specified pathogen-free), 100 g, fasted for 16 h, were injected with 5 mg kg^{-1} reserpine or Ro 4-1284 (i.p.) and decapitated at various times thereafter. Some of the animals received NSD 1015 (100 mg kg^{-1}, i.p., calculated as base) 30 min before death. The rectal temperature of the rats was controlled by insertion of a flexible thermistor. Hypothermia was prevented by keeping the animals in boxes at 28–32°. Determinations of 5-HTP, 5-HT and 5-HIAA in whole brains without cerebellum were carried out using spectrophotofluorimetric methods (Giacalone & Valzelli, 1966; Lindqvist, 1971; Shellenberger & Gordon, 1971; Atack & Lindqvist, 1973). Estimations of 5-HTP were made in a pool of 4 brains, those of 5-HT and 5-HIAA on single brains.

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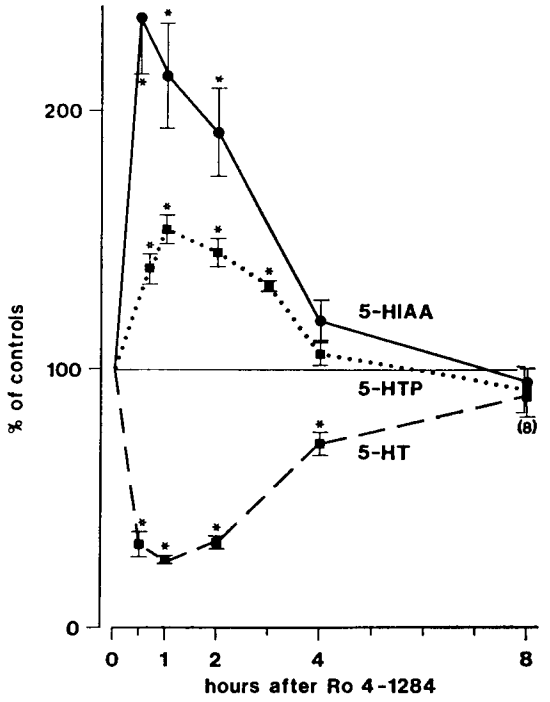

FIG. 1. Effect of Ro 4-1284 (5 mg kg^{-1}, i.p.) on the concentration of 5-hydroxytryptamine (5-HT) (■--■) and 5-hydroxyindoleacetic acid (5-HIAA) (●—●) as well as on the NSD 1015-induced accumulation of 5-hydroxytrytophan (5-HTP) (■. . .■) in rat brains. NSD 1015 (100 mg kg^{-1}) was injected intraperitoneally 30 min before death. Averages ± s.e. of 5-12 experiments. The values are expressed in % of controls (= 100%). Significance *vs* controls: * $P<0.001$ (calculated on absolute values). Absolute values of control (μg g^{-1}): 5-HT = 0·35 ± 0·02, 5-HIAA = 0·54 ± 0·03, 5-HTP: NSD 1015-induced accumulation = 0·17 ± 0·01 (= 100%), without NSD < 0·015.

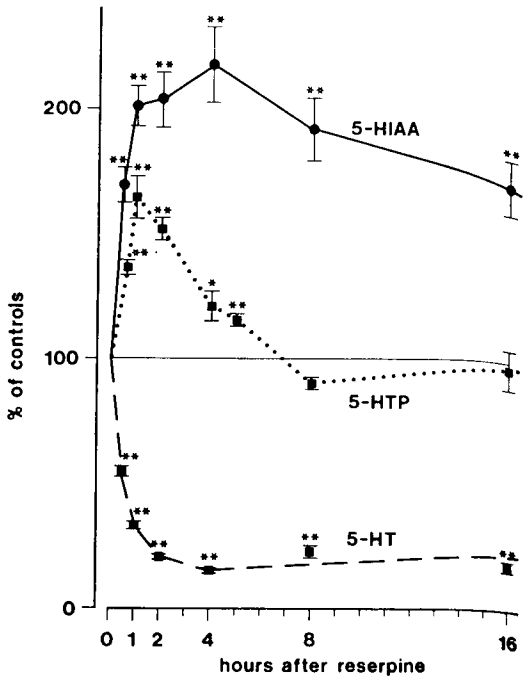

FIG. 2. Effect of reserpine (5 mg kg^{-1}, i.p.) on the concentration of 5-hydroxytryptamine (5-HT) (■--■) and 5-hydroxyindoleacetic acid (5-HIAA) (●—●) as well as on the NSD 1015-induced accumulation of 5-hydroxytryptophan (5-HTP) (■. . .■) in rat brain. NSD 1015 (100 mg kg^{-1}) was injected intraperitoneally 30 min before death. Averages ± s.e. of 6-12 experiments. The values are expressed in % of controls (= 100%). Significance *vs* controls: * $P<0.01$, ** $P<0.001$ (calculated on absolute values). Absolute values of controls (μg g^{-1}): 5-HT = 0·43 ± 0·01, 5-HIAA = 0·55 ± 0·02, 5-HTP: NSD 1015-induced accumulation = 0·17 ± 0·01 (= 100%), without NSD < 0·015.

Figs 1 and 2 indicate the time course of cerebral 5-HTP, 5-HT and 5-HIAA after administration of Ro 4-1284 and reserpine. As expected, 5-HT showed a marked decrease, whereas the concentration of 5-HIAA increased. The action of Ro 4-1284 was relatively short-lasting (about 4–8 h), that of reserpine of long duration (>16 h). Both drugs also enhanced the NSD 1015-induced rise of 5-HTP. After Ro 4-1284 the duration of this enhancement corresponded to the rise of 5-HIAA and to the decrease of 5-HT. In reserpine treated animals, the duration of the enhancement of the 5-HTP rise was longer than that after Ro 4-1284, but much shorter than the duration of the 5-HIAA rise and the 5-HT decrease. The lack of effect of reserpine in enhancing the rise of 5-HTP at times over 5 h after injection of reserpine is in agreement with previous findings. However, the present experiments also show that within a short time (up to about 5 h) after reserpine administration the accumulation of 5-HTP is enhanced.

These results indicate that both Ro 4-1284 and reserpine increase the synthesis of 5-HT. This action may be due to a positive feedback mechanism originating from pre- and/or postsynaptic 5-HT receptors which, owing to depletion of the presynaptic 5-HT stores, are in a hypoactive state. This view is confirmed by preliminary findings in this laboratory that 5-HT receptor stimulants (quipazine, methysergide) (Rodriguez, Rojas-Ramirez & Drucker-Colin, 1973; Kehr, 1977) completely antagonized the enhancement of the 5-HTP rise by reserpine and Ro 4-1284. An activation of 5-HT synthesis as a result of removal of inhibition of tryptophan hydroxylase by end-product cannot, however, be excluded.

The increase of the 5-HIAA concentration after Ro 4-1284 and reserpine is probably due to the metabolism of the 5-HT originating from the 5-HT storage depots (which are relatively rapidly depleted by the drugs) and of the 5-HT formed by enhanced synthesis. Therefore, it

is to be expected that recovery of the cerebral 5-HT content (and thus of the 5-HT storage), return of the 5-HT synthesis to normal and restoration of the 5-HIAA content, would show about the same time course. This was indeed the case for Ro 4-1284. However, with reserpine the enhancement of 5-HTP accumulation induced by NSD 1015 returned to normal long before normal 5-HIAA and 5-HT concentrations were restored. This discrepancy, which also existed when NSD 1015 was replaced by benserazide (800 mg g^{-1}, i.p.), cannot be explained. Reserpine may delay the outflow of 5-HIAA from the brain. This is indicated by previous experiments with probenecid (Tozer & others, 1966). Also, reserpine has been shown to delay the passive outflow of 5-HT in blood platelets (Bartholini, Da Prada & Pletscher, 1965) suggesting that the drug causes alterations in the physico-chemical properties of the plasma membrane. Experiments in this laboratory did not, however, support the hypothesis that reserpine interferes with the outflow of 5-HIAA from the brain. In fact, the rate constant of the decrease of cerebral 5-HIAA ½, 1 and 2 h after administration of the monoamine oxidase (MAO) inhibitor pargyline HCl (Tozer & others, 1966) (100 mg kg^{-1}, i.p., alone or 8 h after 5 mg kg^{-1} reserpine, i.p.) was similar in normal (0·50 h^{-1}, 95% confidence limits 0·60–0·40 h^{-1}) and in reserpinized animals (0·62 h^{-1}, 95% confidence limits 0·75–0·50 h^{-1}). In addition, the probenecid-induced rise of cerebral 5-HIAA (Neff, Tozer & Brodie, 1967) (200 mg kg^{-1} probenecid, i.p., alone or 8 h after 5 mg kg^{-1} reserpine, i.p., death at ½, 1 and 2 h after probenecid) showed a similar increment in normal (0·25 $\mu g\ g^{-1}\ h^{-1}$, 95% confidence limits 0·16–0·35 $\mu g\ g^{-1}\ h^{-1}$) and in reserpinized rats (0·33 $\mu g\ g^{-1}\ h^{-1}$, 95% confidence limits 0·20–0·45 $\mu g\ g^{-1}$ 5-HIAA 8 h after reserpine (Fig. 2) the results favour an enhanced 5-HT turnover due to the drug. Other possibilities have also to be considered, for instance, that 6 and more hours after reserpine injection, decarboxylase inhibitors, for some unknown reason, are no longer able to cause an enhanced accumulation of 5-HTP, although at this time the 5-HT synthesis may still be accelerated. Whether the enhancement of the NSD 1015-induced rise in 5-HTP or the increase of 5-HIAA reflects the true duration of the acceleration of 5-HT synthesis induced by reserpine remains unresolved.

In summary, both Ro 4-1284 (short-acting reserpine-like compound) and reserpine enhanced the 5-HT synthesis in the brain. However, with reserpine the enhancement of 5-HTP accumulation by NSD 1015 was of much shorter duration than the increase in 5-HIAA, whereas with Ro 4-1284 the duration of both these changes was about equal.

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