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EFFECT OF DOPAMINE-β-HYDROXYLASE INHIBITORS 1,1-DIMETHYL-3-PHENYL-2-THIOUREA, FUSARIC ACID AND DIMETHYLDITHIOCARBAMATE, ON THE RAT BRAIN MONOAMINE CONTENT FOLLOWING INTRAVENTRICULAR INJECTION OF L-DOPA

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Abstract—The dopamine-β-hydroxylase inhibitors (DBHI) tested depress the brain noradrenaline content in the brain of normal rats and inhibit the formation of noradrenaline from intraventricularly injected large doses (120 μg) of L-dopa. 1,1-Dimethyl-3-phenly-2-thiourea (U-10,157) and fusaric acid elevate the brain serotonin content in normal, but not in nialamide-pretreated rats. U-10,157 potentiates the depletion of brain serotonin content by L-dopa, whereas fusaric acid and dimethyldithiocarbamate prevent it.

THE BEHAVIOURAL syndrome induced by large intraperitoneal doses of L-3,4-dihydroxyphenylalanine (L-dopa), described for the first time by Blaschko and Chruściel,[1] has been widely applied to study the role of catecholamines in the central nervous system. We reported previously[2] that the injection of microgram quantities of L-dopa into the lateral brain ventricle of the rat also produces a strong behavioural syndrome. However, L-dopa is the precursor of both dopamine and noradrenaline, and to obtain some information about the participation of individual catecholamines in the behavioural reaction observed a more complicated experimental approach is required. The agents often used for this purpose are the inhibitors of dopamine-β-hydroxylase (EC 1.14.2.1., 3,4-dihydroxyphenylethylamine, ascorbate–oxygen reductase hydroxylating (DBHI).

We shall describe here the effects of three relatively new DBHI: 1,1-dimethyl-3-phenyl-2-thiourea (U-10, 157),[3] fusaric acid,[4] and dimethyldithiocarbamate,[5,6] on the brain monoamine levels in rats receiving intraventricularly 120 μg of L-dopa after pretreatment with a monoamine oxidase (EC 1.4.3.4., monoamine–oxygen oxidoreductase (deaminating)) (MAO) inhibitor.

MATERIALS AND METHODS

The experiments were carried out on male Wistar rats, weighing approx. 170 g and having free access to food and water until the injection of a DBHI. The rats were killed by cervical dislocation. Noradrenaline and serotonin were determined in whole brains by the method of Maickel *et al.*,[7] and dopamine, by the method described previously.[8]

Drugs and dosages. L-3,4-Dihydroxyphenylalanine (L-dopa) (Egyt, Budapest) was administered intraventricularly in a dose of 120 μg dissolved in 40 μl of saline. The injection was given to a conscious rat by the method described previously,[8] 3 hr before decapitation in nialamide-pretreated rats, and 75 min before death in reserpinized subjects. Controls rats received 40 μl of saline.

MAO inhibitors; nialamide hydrochloride (prepared from base, "Nuredal", Egyt, Budapest) or pargyline hydrochloride (VEB Fahlberg-List, Magdeburg) were given at doses of 140 or 10 mg/kg, respectively, 18 hr or 30 min before L-dopa.

Reserpine ("Rausedyl", Gedeon Richter, Budapest), 5 mg/kg, was given 22 hr before death.

DBHI: 1,1-Dimethyl-3-phenyl-2-thiourea (Upjohn, Kalamazoo) (U-10,157), 200 mg/kg; fusaric acid calcium complex (Banyu Pharmaceutical Co., Tokyo), 100 mg/kg; sodium dimethyldithiocarbamate (Schuchardt, Munich), 200 mg/kg; they were administered 7, 6 and 5 hr before death, respectively unless otherwise stated. The times of DBHI injections were chosen so that the L-dopa was administered at the maximum of the noradrenaline-depleting effect of the inhibitors.

All compounds other than L-dopa were injected intraperitoneally as solutions in saline (4 or 2 ml/kg), with the exception of U-10,157 and fusaric acid, which were administered as suspensions in 3% solution of Tween 80 in saline.

RESULTS

In normal rats all three DBHI depressed the brain noradrenaline and elevated the brain dopamine levels. (Owing to large variability the elevation of brain dopamine by U-10,157 did not reach the level of statistical significance.) U-10,157 and fusaric acid slightly, but significantly elevated the serotonin brain content (Fig. 1).

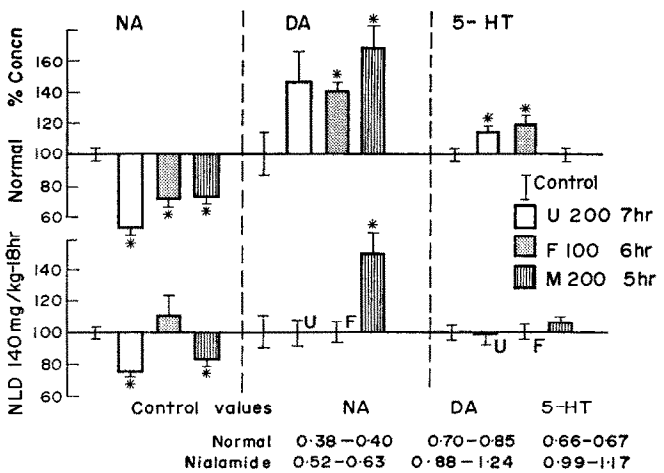

FIG. 1. Whole brain noradrenaline (NA), dopamine (DA) and serotonin (5-HT) in rats given U-10,157 (U), fusaric acid (F), or dimethyldithiocarbamate (M) at specified doses (mg/kg i.p.) and times, without (upper panel) or with nialamide (NLD, 140 mg/kg i.p., 21 hr before death) pretreatment (lower panel). The results are the percentages of control values; each bar represents the mean of 7–10 determinations (± S.E.M.).

* Denote a significant difference from the control value ($P < 0{\cdot}05$, Student's *t*-test).

In nialamide-pretreated rats only U-10,157 and dimethyldithiocarbamate depressed the noradrenaline brain content, and only the latter compound elevated the level of dopamine. None of the DBHI affected the serotonin concentration in the brain of nialamide-pretreated rats (Fig. 1).

In nialamide-pretreated rats 3 hr after intraventricular L-dopa injection the noradrenaline brain level was elevated by 40 per cent, and serotonin level was depressed by 9 per cent. The elevation of noradrenaline was counteracted by all the DBHI tested; the most potent in this respect was U-10,157, which almost completely prevented the rise of brain noradrenaline. U-10,157 potentiated the serotonin-depleting action of L-dopa, whereas dimethyldithiocarbamate effectively prevented the fall of brain serotonin. In rats receiving fusaric acid together with L-dopa the brain serotonin was insignificantly higher than in the groups receiving each of the compounds alone; the results are equivocal, as L-dopa alone in this experiment did not depress the serotonin level (Fig. 2).

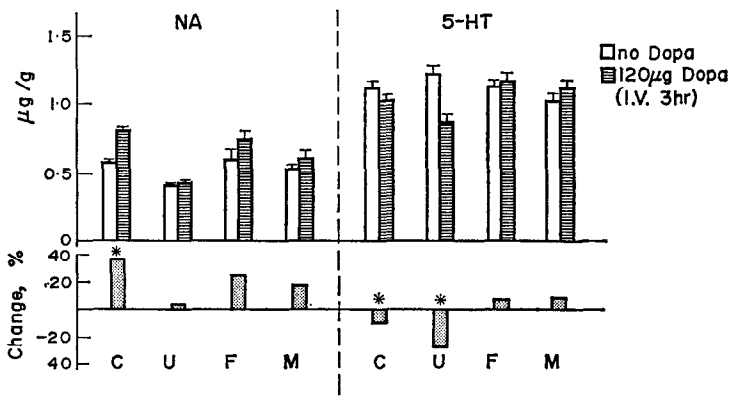

FIG. 2. L-Dopa effects on the whole brain noradrenaline (NA) and serotonin (5-HT) in nialamide-pretreated rats. All rats received intraventricularly saline (40 μl) or L-dopa (120 μg) 3 hr before death. Each bar represents the mean of 7–10 determinations (± S.E.M.) (27–30 determinations in the control group). The per cent changes of the amine content are presented in the lower part.

* The significance of the change ($P < 0{\cdot}05$, Student's *t*-test) (C) Control (solvent); (U) U-10,157, 200 mg/kg i.p. 4 hr before L-dopa; (F) fusaric acid, 100 mg/kg i.p. 3 hr before L-dopa; (M) dimethyldithiocarbamate, 200 mg/kg i.p. 2 hr before L-dopa.

The depression of endogenous brain levels of noradrenaline and serotonin brought about by reserpine was not counteracted by the small dose of pargyline used. In the reserpinized, pargyline-pretreated rats U-10-157 depressed further the noradrenaline brain content, and elevated the level of serotonin. L-Dopa in the reserpine + pargyline treated rats elevated the brain noradrenaline content, and did not change that of serotonin. U-10,157 given before L-dopa inhibited the elevation of brain noradrenaline by 30 per cent, and elevated the brain serotonin (Fig. 3).

DISCUSSION

The inhibition of dopamine-β-hydroxylase should lead to depression of the brain noradrenaline content, and may also produce an increase of brain dopamine, if other

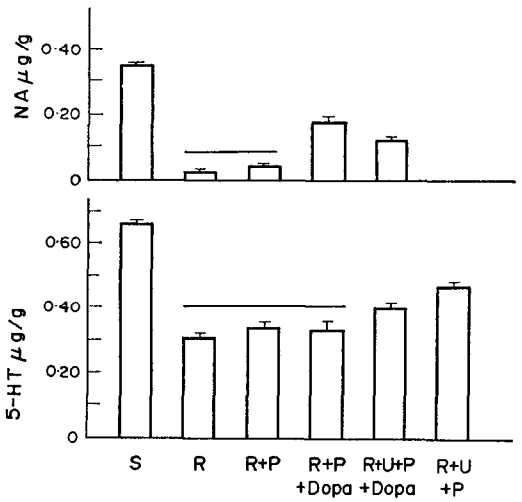

FIG. 3. The effects of L-dopa and U-10,157 on the brain noradrenaline (NA) and serotonin (5-HT) in reserpinized, pargyline-pretreated rats. Each bar represents the mean of 5–10 determinations (± S.E.M.). All the results, with the exception of those below the horizontal bar, differ significantly between themselves ($P < 0{\cdot}05$, Student's *t*-test). (S) solvent; (R) reserpine, 5 mg/kg i.p. 19 hr before L-dopa; (P) pargyline, 10 mg/kg i.p. 30 min before L-dopa; (U) U-10,157, 200 mg/kg i.p. 4 hr before L-dopa; (DOPA) L-dopa, 120 μg intraventricular injection 75 min before death.

mechanisms do not intervene. In normal rats all the three compounds investigated exerted these effects. Our results agree with those reported by others,[3–5,9–12] with the exception that we found an increase in the brain dopamine content after treatment with fursaric acid, whereas Nagatsu *et al.*[9] reported the elevation of the content of this amine only in the adrenals.

On the other hand U-10,157, fusaric acid and dimethyldithiocarbamate differ in their action on brain serotonin, and also in their influence on the catecholamine metabolism in nialamide and L-dopa-treated animals. Dimethyldithiocarbamate is the only one of the compounds investigated which does not change the serotonin brain level, and which elevates brain dopamine in nialamide-pretreated rats. The two remaining DBHI increase the serotonin level in normal, but not in nialamide-pretreated rats, and do not affect the brain dopamine after nialamide pretreatment. Moreover, fusaric acid does not depress the brain noradrenaline in nialamide-pretreated subjects. The depletory action of L-dopa on brain serotonin, is potentiated by U-10,157, but prevented by dimethyldithiocarbamate and, in accordance with literature data,[11] by fusaric acid.

These results suggest that the serotonin turnover and metabolism are affected by DBHI with process not related to dopamine-β-hydroxylase inhibition. It was reported that DBHI of substituted thiourea series increase the brain levels of both serotonin and 5-hydroxyindole-3-acetic acid (5-HIAA),[10] whereas fusaric acid elevates the brain serotonin without affecting 5-HIAA level,[11] and dimethyldithiocarbamate influences neither brain serotonin, nor 5-HIAA.[13] It seems, therefore, that only U-10,157 and related thioureas increase the turnover of brain serotonin.

Although U-10,157 does not affect the brain serotonin after nialamide pretreatment,

it still elevates the level of this amine in reserpinized rats in spite of MAO inhibition by pargyline. The lack of action of U-10,157 on brain serotonin in nialamide-pretreated animals is, therefore, related probably to the change in the serotonin turnover under the conditions of an elevated steady-state level of this amine in the brain.[14] It might be noted that several agents which elevate brain serotonin in normal animals fail to exert this effect after nialamide pretreatment.[15,16] A similar explanation may be offered for the lack of action of U-10,157 and fusaric acid on the dopamine brain content in nialamide-pretreated rats, although the effect of dimethyldithiocarbamate suggests that some other factors may interfere.

Johnson *et al.*[10] have advanced the hypothesis about the interdependence between the brain noradrenaline and serotonin systems. As a consequence of this interdependence the depression of noradrenaline synthesis by a DBHI should increase the central serotonin level and/or turnover. However, we have found no correlation between the brain noradrenaline and serotonin levels in rats receiving U-10,157 alone ($r = -0{\cdot}07$), and even positive (if insignificant) correlation in the group treated with fusaric acid ($r = +0{\cdot}30$). The results obtained with dimethyldithiocarbamate also contradict the assumption of the existence of a negative correlation between the brain levels of noradrenaline and serotonin after the inhibition of dopamine-β-hydroxylase.

The DBHI investigated effectively inhibited the increase of brain noradrenaline after the intraventricular injection of L-dopa in nialamide-pretreated rats, and U-10,157, which completely prevented this increase, also inhibited (by 30 per cent) the noradrenaline elevation brought about by L-dopa in the rats pretreated with reserpine and pargyline.

It seems, therefore, that DBHI inhibit considerably the biotransformation of L-dopa to noradrenaline under the conditions of the experiment. Owing to that they may possibly serve as a tool for the assessment of the relative participation of the newly formed dopamine and noradrenaline in the behavioural syndrome induced by the intraventricular injection of L-dopa.[2]

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