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The effect of simultaneous administration of 3,4-dihydroxyphenylpyruvic acid and L-dopa on the bioavailability of L-dopa in rat and mouse

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In the rat, administration of 3-(3,4-dihydroxy)-L-phenylalanine (L-dopa) simultaneously with the corresponding α-keto acid, 3,4-dihydroxyphenylpyruvic acid (DHPPA), gives significantly higher concentrations of L-dopa in the serum and of dopamine and homovanillic acid in the brain than the same dose of L-dopa alone. Correspondingly, DHPPA potentiates the effect of L-dopa on the locomotor activity in reserpine-treated mice. DHPPA is postulated to increase the absorption of intact L-dopa from the gut by preventing the transamination reaction between L-dopa and the physiological α-keto acids.

3-(3,4-Dihydroxy)-L-phenylalanine (L-dopa) is now widely used in the therapy of parkinsonism. The rationale of the treatment is to compensate the diminished dopamine (DA)-forming capacity in the basal ganglia of the brain by oral administration of the DA precursor L-dopa, which penetrates the blood-brain barrier and is decarboxylated to DA in the brain.

Orally administered L-dopa is mainly metabolized in the gastrointestinal tract (Rivera-Calilim et al 1970; Cotler et al 1976). Only 20–30% reaches the systemic circulation and less than 5% the central nervous system.

One of the main catabolic reactions of L-dopa is the decarboxylation to DA, which is further oxidized by monoamine oxidases to 3,4-dihydroxyphenylacetic acid (DOPAC) and methylated to homovanillic acid (HVA). Another metabolic pathway of L-dopa is transamination or deamination to 3,4-dihydroxyphenylpyruvic acid (DHPPA), followed either by oxidative decarboxylation to DOPAC or hydrogenation and methylation to vanillylactic acid (VLA). HVA is the main accumulating metabolite of DA and its concentration in the brain may be held as an indicator of the metabolic turnover of DA in this tissue. Fig. 1 presents the relations between the most important decarboxylation and transamination products of L-dopa.

Transamination with physiological α-keto acids as a possible cause of L-dopa losses in connection with absorption of the drug, has received relatively little attention. If the transamination reaction plays any role in the intestinal losses of L-dopa, then the addition of the reaction product DHPPA would have an L-dopa-sparing effect by forcing the equilibrium of the transamination reaction in the L-dopa-forming direction.

In this paper the synergistic effect of simultaneous administration of DHPPA and L-dopa on serum L-dopa concentration as well as brain DA and HVA concentrations in the rat and on the locomotor activity in reserpine-treated mice is reported.

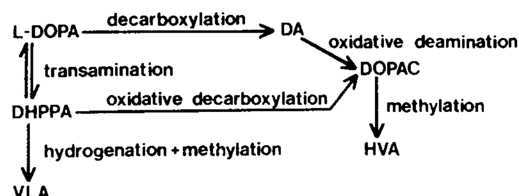

FIG. 1. The relations between the most important decarboxylation and transamination products of L-dopa.

MATERIAL AND METHODS

Recording of motor activity in mice

Male mice (NMRI strain), 18–22 g, were used. The motor activity was measured in groups of 10 mice on an Animex activity meter. The activity was recorded every 15 min for 2½ h. All animals were pretreated with reserpine, 10 mg kg^{-1} i.p. 18 h before the oral administration of the test drugs.

The compounds tested were: L-dopa (500 mg kg^{-1}), DHPPA (500 mg kg^{-1}), L-dopa + DHPPA (500 +200 mg kg^{-1}). The drugs were suspended in a 5% solution of gum arabic. The control animals received a corresponding volume of the vehicle. Recording of the motor activity started immediately after administration of the drugs.

* Correspondence.

Determination of L*-dopa in rat serum*

Animals. Sprague–Dawley male rats, 230–280 g, were fasted 18 h before the oral administration of the drugs and had free access to water. The compounds tested were: L-dopa (200 mg kg^{-1}), DHPPA (200 mg kg^{-1}), L-dopa + DHPPA (200 + 200 mg kg^{-1}). The drugs were suspended in a 5% gum arabic solution. The control animals received the corresponding volume of the vehicle. Blood samples were taken from the tail artery 10, 20 and 40 min after the drug. The serum samples were kept at −20 °C until they were assayed for L-dopa.

Precipitation of proteins. The proteins in the serum were precipitated with $HClO_4$ (4M). The tubes were shaken vigorously and were allowed to stand at +4 °C for 1 h, after which the samples were centrifuged for 10 min at 10000 *g*. 1·0 ml 0·1M citrate buffer (pH 4·0) and 1·0 ml H_2O were added to 0·5 ml of the supernatant. The pH of the solution was adjusted to 2·0 ± 0·2 with 1M HCl or 1M NaOH.

Isolation of L*-dopa from the supernatant.* The separation of L-dopa from the serum was on Dowex 50W columns according to Curzon et al (1972).

Fluorometric assay of L*-dopa.* The fluorophore of L-dopa was prepared and assayed by the method of Geissbuehler (1973).

Determination of DA and HVA in rat brain

Animals. Sprague–Dawley male rats 230–280 g were fasted for 18 h before the oral administration of the drugs and had free access to water. The compounds tested were L-dopa, DHPPA, and L-dopa + DHPPA. The substances were suspended in a 5% solution of gum arabic. The control animals were given a corresponding volume of the vehicle. The rats were decapitated and the brains quickly removed 30, 60, 90 and 180 min after the substances were given. The brains were weighed and kept at −20 °C (not more than 7 days) until they were analysed for DA and HVA.

Extraction of DA and HVA. A modification of the method described by Haubrich & Denzer (1973) was used for the extraction. The rat brains were homogenized in a Sorval homogenizer in 15·0 ml of cold acidified (10 mM HCl) butanol. The homogenate was centrifuged for 20 min at 20000 *g*. 4·0 ml of the supernatant was added to a tube containing 10·0 ml iso-octane and 1·5 ml 0·01 M HCl. The tube was shaken for 10 min and centrifuged for 15 min at 2000 *g*. The organic phase was used for the HVA assay and the aqueous phase for the DA assay.

Assay of DA. 1·0 ml of the DA-containing aqueous phase was added to a tube containing 200 mg of Al_2O_3 (prepared as described by Anton & Sayre 1962) and 1·5 ml of tris-HCl buffer (0·5 M, pH 8·5). The tubes were shaken for 10 min and centrifuged for 5 min at 1000 *g*. The supernatant was removed and the Al_2O_3 washed with 2·0 ml H_2O. DA was eluted from the Al_2O_3 with 2·2 ml phosphate buffer (0·5 M, pH 6·5), by shaking for 10 min. The sample was centrifuged for 5 min at 1000 *g*. 2·0 ml of the supernatant was analysed fluorometrically according to Chang (1964).

Assay of HVA. 12 ml of the organic phase, containing HVA, was shaken for 10 min with 3·5 ml tris-HCl buffer (0·05 M, pH 8·5) and centrifuged for 5 min at 2000 *g*. The organic phase was removed and 3·0 ml of the aqueous phase was used for the fluorometric determination of HVA by the procedure of Andén et al (1963).

RESULTS

Fig. 2 shows the locomotor activity in reserpine-treated mice (10 mg kg^{-1} i.p.) after single oral doses of L-dopa 500 mg kg^{-1}, DHPPA 500 mg kg^{-1} and L-dopa + DHPPA 500 + 200 mg kg^{-1}. L-Dopa stimulates the locomotor activity while DHPPA alone has no effect. Simultaneous administration of L-dopa and DHPPA potentiates the activity significantly compared with L-dopa alone.

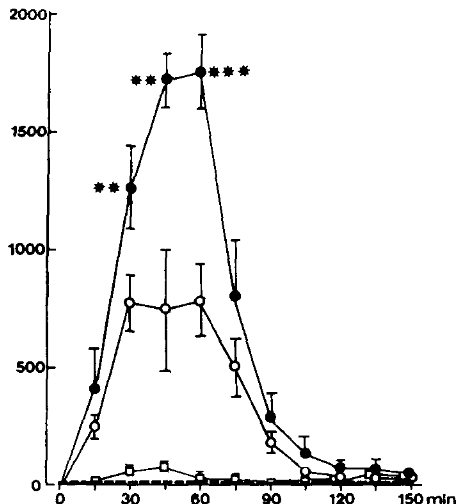

FIG. 2. Locomotor activity in reserpine-treated mice after single oral doses of L-dopa 500 mg kg^{-1} (○), DHPPA 500 mg kg^{-1} (□) and L-dopa + DHPPA (500 mg kg^{-1} + 200 mg kg^{-1}) (●). Control = (– –). Ordinate: counts/15 min. Abscissa: time (min). Each value is the mean ± s.e.m. of 6 observations. The levels of significance refer to the difference from the L-dopa-treated mice at the same time point: ** $P < 0·01$: *** $P < 0·001$.

L-Dopa concentrations in rat serum at different times after single oral doses of L-dopa (200 mg kg^{-1}), DHPPA (200 mg kg^{-1}) and L-dopa + DHPPA (200 +200 mg kg^{-1}) are presented in Fig. 3. L-Dopa and DHPPA administered separately caused almost identical L-dopa concentration time curves. When used in combination, the drugs give serum L-dopa concentrations which far exceed their additive effect.

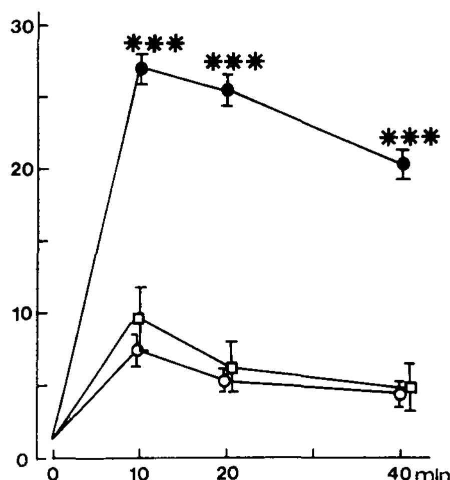

FIG. 3. L-Dopa concentrations in rat serum after single oral doses of L-dopa (200 mg kg^{-1} (○), DHPPA 200 mg kg^{-1}) (□) and L-dopa + DHPPA (200 mg kg^{-1} + 200 mg kg^{-1}) (●). Each value is the mean ± s.e.m. of 6 observations. Ordinate: L-dopa μg ml^{-1} serum. Abscissa: time (min). The levels of significance refer to the difference from the L-dopa-treated rats at the same time point: *** $P < 0.001$.

Fig. 4 shows that DHPPA also enhances the L-dopa-induced rise of cerebral DA and HVA in the rat. DHPPA administered alone has a negligible effect on both DA and HVA concentrations in the brain.

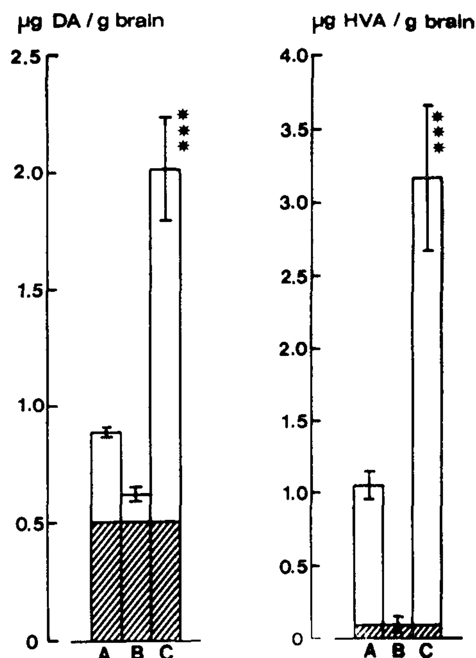

FIG. 4. DA and HVA concentrations in the rat brain 60 min after a single oral dose of L-dopa 200 mg kg^{-1} (A), DHPPA 200 mg kg^{-1} (B) and L-dopa + DHPPA (200 mg kg^{-1} + 200 mg kg^{-1}) (C). The hatched areas show the endogenous concentrations of DA and HVA. Ordinate: μg g^{-1} brain. Each value is the mean of 4–6 observations. Significantly higher levels than obtained from L-dopa-treated rats: *** $P < 0.001$.

DISCUSSION

It has been shown that cerebral accumulation of DA causes locomotor stimulation in mice (Bartholini et al 1969; Andén et al 1973). In our studies DHPPA given together with L-dopa enhanced the locomotor stimulation induced by the latter, while alone it had no effect on the motor activity. We confirmed these results in experiments with rats, in which we could show that the DA and HVA values in brain were significantly higher after simultaneous administration of L-dopa and DHPPA than after L-dopa alone. Correspondingly, a much higher L-dopa concentration in the blood was attained by combination of the substances, which means that there is in the systemic circulation more amino acid available for the brain. Shindo & Maeda (1974) showed in vitro the transformation of DHPPA to L-dopa by tissue homogenates, and thereafter (1977) studied the possibility of using DHPPA as a brain dopamine precursor. They came to the conclusion that DHPPA is not a good replacement for L-dopa, mainly owing to its slow absorption from the intestinal tract. Likewise, we could not see any central effects of oral doses of DHPPA on rats and mice. However, in the rat, DHPPA increased the blood concentration of L-dopa to the same level as the equivalent dose of L-dopa. Why the L-dopa formed after an oral dose of DHPPA does not penetrate the blood-brain barrier is not clear. One possibility is that there is an excess of DHPPA in the circulation, which inhibits the transport of L-dopa through the blood-brain barrier.

Sandler (1974) studied the metabolites of L-dopa excreted into the urine and concluded that transamination to DHPPA followed by hydrogenation and methylation to VLA was the main catabolic pathway of L-dopa when the decarboxylation reaction was blocked by hydrazine-derived decarboxyla-

tion inhibitors. Fellman et al (1976) demonstrated that the hydrazine-derived decarboxylation inhibitors also inhibit tyrosine aminotransferase of mammalian liver, and Shindo & Maeda (1974) found that the formation of L-dopa in vivo from D-dopa proceeds through deamination of D-dopa to DHPPA and amination of this keto acid to L-dopa.

We propose tentatively that the L-dopa-sparing action of DHPPA could be due mainly to the prevention of the transamination reaction between L-dopa and physiological α-keto acids, although inhibition of the decarboxylation of L-dopa and of its methylation to 3-*O*-methyldopa, by depletion the active methyl group pool, may be operating as well.

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