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Effects of 3,4-dihydroxyphenylpyruvic acid and L-glutamic acid on some pharmacokinetic parameters of L-dopa in the rat

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In the rat, various oral doses of 3,4-dihydroxyphenylpyruvic acid (DHPPA) administered simultaneously with L-dopa were shown to elevate the serum L-dopa and cerebral dopamine concentrations dose-dependently. With increasing DHPPA: L-dopa ratio the L-dopa-sparing effect became greater. Although oral and intravenous doses of DHPPA showed that it was readily converted to L-dopa in the serum, only small amounts of dopamine (DA) were detected in the brain. The high 3-*O*-methyldopa concentrations, measured in the serum after DHPPA administration, might be responsible for the failure of the L-dopa formed to pass the blood-brain barrier. Concomitant administration of L-dopa and glutamic acid gave significantly higher L-dopa values in the serum and DA and homovanillic acid values in the brain than did the same dose of L-dopa alone. This indicates that the transamination of L-dopa can also be effected by coadministration of L-dopa and an amino group donor in vivo.

Previously Hietala et al (1979) have shown that in the rat, concomitant oral administration of L-3,4-dihydroxyphenylanine (L-dopa) and the corresponding α-keto acid, 3,4-dihydroxyphenylpyruvic acid (DHPPA) caused significantly higher serum concentrations of L-dopa and brain concentrations of dopamine (DA) and homovanillic acid (HVA) than did the same dose of L-dopa alone. It was proposed that the increased bioavailability of L-dopa is mainly due to an increased absorption of intact L-dopa through prevention of the transamination reaction, which converts L-dopa to its physiological α-keto acid. If the effect of DHPPA on the bioavailability of L-dopa is caused by prevention of the transamination reaction, it seems logical that coadministration of an amino acid which acts as amino group donor in the amination of DHPPA, also should force the reaction in the L-dopa forming direction, according to the law of mass action. Maeda et al (1977) investigated in vitro the ability of various amino acids to act as amino group donors in the amination of DHPPA in rat liver. Of the tested drugs L-glutamate, L-tryptophan and L-phenylalanine were found to be effective.

The purpose of this study was to obtain more detailed information on the L-dopa-sparing effect of DHPPA and to judge whether the bioavailability of L-dopa is increased by concomitant administration of glutamate.

MATERIALS AND METHODS

Determination of serum L-dopa and 3-O-methyldopa (3O-MD) and cerebral dopamine (DA) and homovanillic acid (HVA)

Male Sprague-Dawley rats, 230–280 g, were fasted 18 h before the oral and intravenous administration of the drugs. The rats had free access to water. The compounds tested: L-dopa, DHPPA, L-dopa + DHPPA, L-dopa + L-glutamate, were administered in various doses. The drugs were suspended in a 5% gum arabic solution for oral administration and dissolved in 0·9% NaCl (saline) containing 0·001 M HCl for the intravenous route. The control animals received a corresponding volume of the vehicle. Blood samples were taken from the tail artery 10, 20 and 40 min after the administration of the drug. For the determination of DA the rats were decapitated 60 min after the substances were given, and the brains were quickly removed. The serum and brain samples were kept at −20 °C until analysed. The areas under the time concentration curves of L-dopa (AUC) were calculated by the trapezoidal rule.

Fluorometric assays of L-dopa, DA, HVA and 3-OMD

For L-dopa, DA and HVA, the samples were prepared and assayed according to Hietala et al (1979). Modifications of the methods described by Andén et

al (1963) Murphy et al (1969) and Kim et al (1977) were used for the extraction and assay of 3-OMD.

Precipitation of proteins. The proteins in the serum were precipitated with $HClO_4$ (4 M). The tubes were shaken vigorously, allowed to stand at +4 °C for 15 min and centrifuged for 10 min at 10 000 *g*.

Extraction of 3-OMD. Supernatant (2·0 ml) was added to a tube containing 11·0 ml of n-butylacetate. The tube was shaken for 10 min and centrifuged for 15 min. The aqueous phase was washed with 5·5 ml of n-butylacetate.

Assay of 3-OMD. To 1·0 ml of the aqueous phase was added 1·0 g of Al_2O_3 (prepared as described by Anton & Sayre 1962), 2·0 ml of 0·2 M ammonium acetate-buffer and 0·5 ml of 1 M Na_2CO_3. The pH was adjusted to 8·45–8·55 with 1 M NaOH or 1 M HCl. The samples were shaken vigorously for 10 min and centrifuged at 2000 *g*. 2·0 ml of 10 M NH_3 solution was added to 2·0 ml of the supernatant, shaken and 0·4 ml of 0·01% K_3 $Fe(CN)_6$ solution added. 4 min later the reaction was stopped by addition of 0·4 ml of 0·1% cystein solution. The fluorescence was measured at an excitation wavelength of 400 nm and an emission wavelength of 510 nm.

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. The animals were pretreated with reserpine, 10 mg kg^{-1} i.p., 18 h before the intravenous administration of the test drugs.

20–30% of orally administered L-dopa is recovered in the blood. The doses in this intravenous study were 25% of the oral doses used in a previous study (Hietala et al 1979), in order to make the two studies more comparable. The compounds tested were: L-dopa (125 mg kg^{-1}), DHPPA (125 mg kg^{-1}), L-dopa + DHPPA (125 + 125 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 drug.

Because the locomotor activity is dependent on the DA concentration in the brain (Bartholini et al 1969) this method can be seen as an indirect means for evaluating the effect of the drugs on cerebral DA.

RESULTS

The areas under the L-dopa concentration-time curves (AUC) after single oral doses of L-dopa 50, 100, 200 mg kg^{-1}, DHPPA 50, 100, 200 mg kg^{-1}, L-dopa + DHPPA 50 + 50, 50 + 100, 50 + 200 mg kg^{-1} and L-dopa + DHPPA 100 + 100, 100 + 200, 100 + 300 mg kg^{-1} are presented in Fig. 1. L-Dopa and DHPPA, given separately or combined, caused a dose-dependent elevation of the serum L-dopa concentration. The L-dopa concentration reached a plateau after a dosage of L-dopa 100 + DHPPA 300.

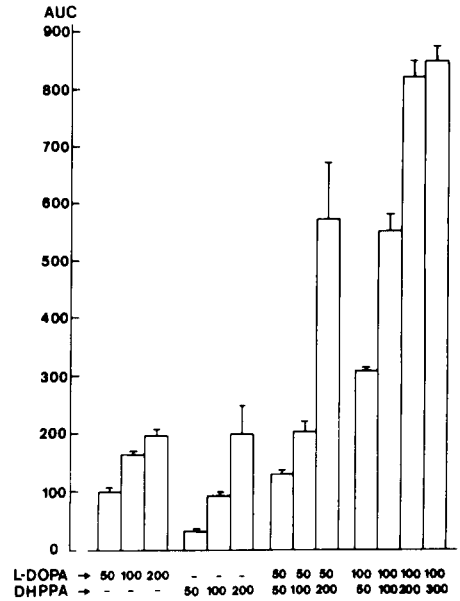

FIG. 1. Area under curve (AUC) calculated from L-dopa concentration time curves after single oral doses of L-dopa, DHPPA and L-dopa + DHPPA. Each value is the mean ± s.e.m. of 4–6 observations.

Fig. 2 shows that, when the cerebral DA was measured 1 h after the oral administration of the above substances and doses, a very good correlation with the corresponding serum L-dopa concentrations was attained. The only exception was the effect of DHPPA alone. DHPPA caused only a negligible elevation of DA and no dose-dependency was found within the used dose range.

After intravenous administration of equal doses of L-dopa and DHPPA separately, almost identical AUC were attained, while simultaneous administration of the two substances had an additive effect on the serum L-dopa concentration (Fig. 3A). Significantly ($P < 0.001$) more DA was measured in the brain after the combination than after intravenous doses of L-dopa or DHPPA alone. The rise was more than additive. DHPPA had a small effect on the cerebral DA concentration (Fig. 3B).

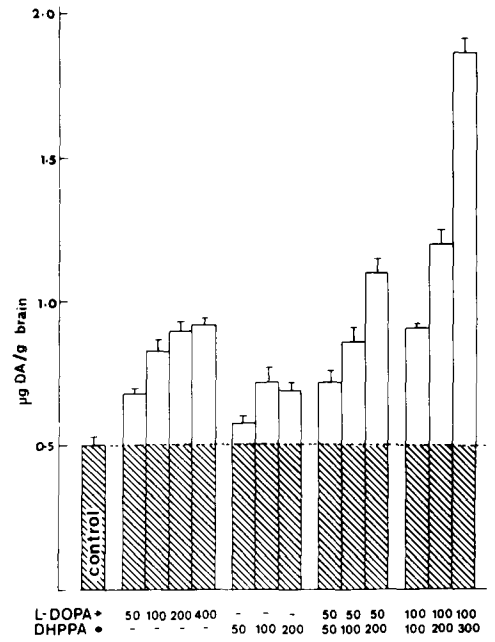

FIG. 2. DA concentrations in the rat brain 60 min after single oral doses of L-dopa, DHPPA and L-dopa + DHPPA. Ordinates: μg^{-1} brain. Each value is the mean of 4–6 observations.

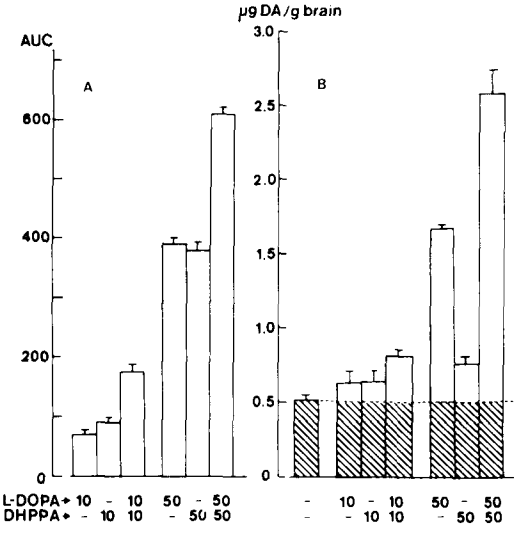

FIG. 3. A. Area under curve (AUC) calculated from L-dopa concentration time curves and B. DA concentrations in the rat brain 30 min after intravenous doses of L-dopa, DHPPA and L-dopa + DHPPA. Each value is the mean ± s.e.m. of 6 observations.

Fig. 4 shows the locomotor activity in reserpine-treated mice (10 mg kg^{-1} i.p.) after single intravenous injections of L-dopa 125 mg kg^{-1}, DHPPA 125 mg kg^{-1} and L-dopa + DHPPA 125 + 125 mg kg^{-1}. DHPPA had no effect on the locomotor activity, while the combination of L-dopa and DHPPA enhanced the activity significantly compared with L-dopa alone.

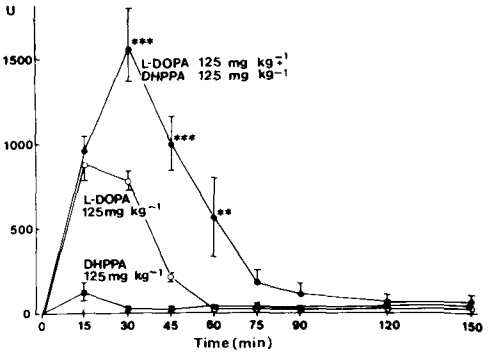

FIG. 4. Locomotor activity in reserpine-treated mice after intravenous injections of L-dopa 125 mg kg^{-1} ○, DHPPA 125 mg kg^{-1} ■ and L-dopa + DHPPA 125 + 125 mg kg^{-1} ●. Ordinate: counts/15 min. Abscissa: time (min). Each value is the mean ± s.e.m. of 6 observations. The levels of significance refer to the difference from the L-dopa-treated mice at the same time point: $P < 0.05$, $P < 0.001$.

Fig. 5 shows the time-concentration curves of 3-OMD in the serum after oral doses of L-dopa, DHPPA and L-dopa + DHPPA. L-Dopa caused a negligible elevation of 3-OMD, while DHPPA elevated the concentration as much as did the combination.

The time concentration curves of serum L-dopa and cerebral DA and HVA after oral doses of L-dopa and L-dopa + glutamate to rats are shown in Fig. 6. The combination elevates all parameters significantly more than L-dopa alone.

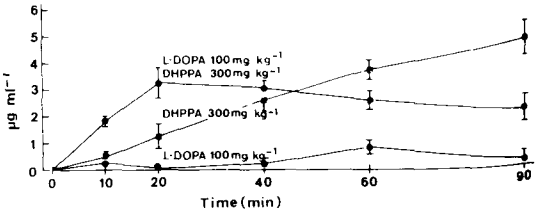

FIG. 5. 3-OMD concentrations in rat serum after single oral doses of L-dopa 100 mg kg^{-1}, DHPPA 300 mg kg^{-1} and L-dopa + DHPPA 100 + 300 mg kg^{-1}. Each value is the mean ± s.e.m. of 6–10 values. Ordinate: 3-OMD μg ml^{-1} serum. Abscissa: time (min).

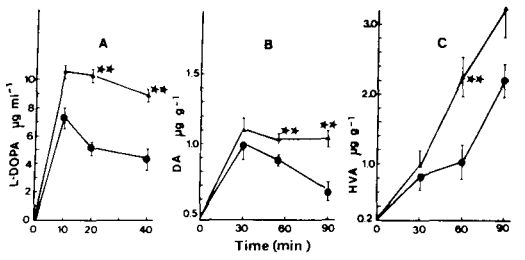

FIG. 6. A. L-Dopa concentrations in rat serum B. DA concentrations in brain C. HVA concentrations in brain at different times after oral administration of L-dopa 200 mg kg^{-1} ○ and L-dopa + glutamate 200 + 300 mg kg^{-1} ▲ to rats. Each value is the mean ± s.e.m. of 4–6 values. The levels of significance refer to the difference from the L-dopa-treated rats at the same time point: $P < 0.01$.

DISCUSSION

L-Dopa administered orally to rats at doses of 50, 100 and 200 mg kg^{-1} increased the serum L-dopa and cerebral DA concentrations dose-dependently. The correlation between corresponding L-dopa and DA values was good. Orally administered L-dopa has a low bioavailability, owing to extensive gastrointestinal metabolism (Rivera-Calimlim et al 1971; Andersson et al 1975).

A dose-dependent increase in serum L-dopa was also demonstrated after oral administration of DHPPA. Since the gastrointestinal absorption of DHPPA is very slow (Maeda et al 1977), it is obvious that most of the L-dopa found in the serum after oral DHPPA administration originates from DHPPA transaminated to L-dopa in the gastrointestinal tract. Although the serum L-dopa profiles were almost identical after oral doses of L-dopa and of DHPPA, only a negligible increase in cerebral DA concentration was seen after DHPPA. No dose-dependency was seen. Consistent results, i.e., high serum L-dopa and low cerebral DA concentrations, were attained after intravenous administration of DHPPA to rats. Likewise, intravenous administration of DHPPA to mice had almost no effect on the locomotor activity.

In the present investigation the serum concentration of 3-OMD, a major L-dopa metabolite, after DHPPA administration was as high as after the combination of L-dopa and DHPPA. Maeda et al (1977) found that 3-OMD represented 33% of all L-dopa metabolites in the brain after intravenous administration of DHPPA-2-^{14}C to rats. L-Dopa and 3-OMD are transported through the blood-brain barrier by similar mechanisms, but 3-OMD has greater affinity for the transport carrier (Wade et al 1976). The failure of L-dopa, converted from DHPPA after intravenous or oral administration, to penetrate the blood-brain barrier might therefore be due to high concentrations of 3-OMD in the systemic circulation.

Simultaneous administration of L-dopa and various doses of DHPPA caused a dose-dependent increase in serum L-dopa and cerebral DA concentrations. With increasing DHPPA : L-dopa ratio the L-dopa-sparing effect became greater, far exceeding the additive effect of these two drugs. The results indicate that the ability of DHPPA to force the equilibrium of the transamination reaction in the L-dopa-forming direction is highly dependent on the DHPPA : L-dopa ratio. The L-dopa : 3-OMD ratio in the serum was much higher after coadministration of the drugs than after DHPPA alone. The penetration of L-dopa into the brain is therefore not likely to be prevented by 3-OMD after coadministration of L-dopa and DHPPA.

Concomitant intravenous administration of L-dopa and DHPPA to rats caused a significantly greater increase of DA in the brain than intravenous administration of L-dopa alone. Amination of DHPPA has been demonstrated not only in intestinal homogenates but also in liver, kidney and brain homogenates (Fonnum et al 1964; Maeda et al 1977). It is therefore probable that the higher concentrations of DA in the brain are due to prevention of the transamination reaction in the systemic circulation.

When L-dopa was administered together with an amino acid postulated to act as an amino group donor in the transamination reaction, more L-dopa reached the systemic circulation and higher DA and HVA concentrations were measured than after L-dopa alone. This supports the assumption that the equilibrium of the transamination reaction is forced in the L-dopa-forming direction not only by coadministration of the corresponding keto acid but also by an amino acid which can act as an amino group donor. Maeda et al (1977) have shown in vitro that various amino acids can act as amino group donors in the conversion of DHPPA to L-dopa, and they also found that DHPPA is readily aminated in various rat tissues without additional extragenous amino group donors. Thus, there should normally be endogenous amino group donors available for amination in the organism.

In conclusion, an increased bioavailability of L-dopa was attained by coadministration of either the corresponding keto acid, which acts as an amino group acceptor, or an amino acid acting as an amino

group donor in the transamination reaction of L-dopa.

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REFERENCES

Andersson, I., Granerus, A.-K., Jagenburg, R., Svanberg, A. (1975) Acta Med. Scand. 198: 415–420

Andén, N.-E., Roos, B. E., Werdinius, B. (1963) Life Sci. 7: 448–458

Anton, A., Sayre, D. (1962) J. Pharmacol. Exp. Ther. 138: 360–375

Bartholini, G., Blum, J. E., Pletscher, A. (1969) J. Pharm. Pharmacol. 21: 297–301

Fonnum, F., Haaraldsen, R., Tangen, O. (1964) J. Neurochem. 11: 109–118

Hietala, P., Lindén, I.-B., Grönfors, N. (1979) J. Pharm. Pharmacol. 31: 205–208

Kim, B. K., Koda, R. T. (1977) J. Pharm. Sci. 66: 1632–1634

Maeda, T., Miyakoshi, N., Shindo, H. (1977) Chem. Pharm. Bull. 25: 2001–2012

Maeda, T., Shindo, H. (1977) Ibid. 25: 1992–2000

Murphy, G.-F., Robinson, D., Sharman, F. (1969) Br. J. Pharmacol. 36: 107–115

Rivera-Calimlim, L., Dujovne, C. A., Morgan, J. A., Lasagna, C., Bianchine, J. K. (1971) Eur. J. Clin. Invest. 1: 313–320

Wade, L. A., Katzman, R. (1976) Life Sci. 17: 131–136