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Dopaminergic metabolism in various rat brain areas after L-dopa loading

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The time course of dopamine (DA), 3,4-dihydroxyphenylacetic acid (DOPAC), homovanillic acid (HVA), 3-methoxytyramine (3-MT) and 3-methoxytyrosine (3-*O*-Medopa) concentrations in rat brain after treatment with L-dopa + benserazide has been investigated in the striatum, hypothalamus, hippocampus and cerebellum. These areas were selected for their different dopaminergic activities. After L-dopa loading, DA, DOPAC and HVA were increased in all the structures, but the largest increases were in those tissues with the less dopaminergic activity, while 3-MT increased in the hypothalmus, hippocampus and cerebellum but was lowered in striatum. 3-*O*-Medopa, which is the direct product of the *O*-methylation of L-dopa, did not show any specific distribution. The data provide evidence that the striatum, by feed-back mechanisms and specific enzymatic activity, is able to ensure a better regulation of dopaminergic activity than the other structures, thereby overcoming excess L-dopa.

Lammers & Van Rossum (1968) reported a 'bizarre social behaviour' which occurs after treating male rats with the combination L-dopa and benserazide. Some of the components of this behavioural syndrome could be classed as emotional aggression which is known to have a complex biochemical basis (Reis 1974).

We have previously studied the comportmental effects of such a treatment (El Gemayel et al 1986) which were suggested to be related to different perturbations of the monoamine metabolism after L-dopa loading (Deakin & Dashwood 1981).

The biochemical study of the effects of treatment with L-dopa and benserazide was aimed at elucidating the mechanism of action of L-dopa in the treatment of Parkinson's disease (Bartholini et al 1971). In those studies, fluorimetric and radioactive methods were used to quantify endogenous catecholamine levels (Calne et al 1969). Many explanations were proposed to elucidate this mechanism and the biochemical and comportmental disorders caused by the L-dopa therapy (Hefti et al 1981), however this field has remained little known until recently (Ponzio et al 1984).

We earlier used a chromatographic method with electrochemical detection to study the striatal metabolism of dopamine after L-dopa loading and we found that the simultaneous evaluation of the time course of dopamine (DA), 3,4-dihydroxyphenylacetic acid DOPAC, homovanillic acid (HVA) and 3-methoxytyramine (3-MT) levels over 9 h in this dopaminergic structure showed that there was a dramatic increase in the concentrations of these compounds and then they slowly disappeared. Furthermore, 3-methoxytyrosine (3-*O*-Medopa), an unusual metabolite, was detected in very high concentration (El Gemayel et al 1986). We therefore have undertaken a further study of the evolution of the levels of these five compounds in four brain areas having different dopaminergic innervation. Striatum was selected as the main dopaminergic area, the hypothalamus for its numerous neurotransmitter interactions, the hippocampus as a minor dopaminergic region and the cerebellum as an area completely deprived of DA terminals and perikarya.

Materials and methods

Eighty male Sprague-Dawley rats (Charles Rivers, France) (225–250 g) housed under standard conditions were used. Treatments were randomly attributed according to a 'balanced lattice design' (Cochran & Cox 1957). Treated animals were injected with L-dopa

* Correspondence.

(Roche) (125 mg kg^{-1} i.p. route), 30 min after pretreatment with benserazide (Roche) (50 mg kg^{-1} i.p. route), and control rats received i.p. 0·9% NaCl (saline) injections.

At 0·5, 1, 1·5, 3, 4·5, 6, 7·5 and 9 h after L-dopa administration the animals were decapitated, the brain rapidly removed and hypothalamus, hippocampus, striatum and cerebellum isolated within 10 min, on a plate chilled to −15 °C. The structures were stored at −80 °C in capped polyethylene tubes until assay. For each time, five treated and five control rats were killed. This was to take into account eventual circadian changes on the levels of DA and its related metabolites. Every experiment was begun at 0930 h. However, in untreated groups, because of the slight circadian variations, means were calculated by pooling all control values.

Biochemical determinations were performed according to the method of El Gemayel et al (1986) which consisted of a chromatographic separation of the homogenized cerebral sample followed by an electrochemical detection.

Results

In our experimental conditions, the dopaminergic compounds investigated (DA, DOPAC, HVA, 3-MT) were not detectable in all cases, depending on the treatment and tissue concentrations. In the hypothalamus and striatum, DA and its classical metabolites were detected both in control and treated rats thereby allowing the calculation of the data as a percentage of variations versus control. In the hippocampus and cerebellum, DA, DOPAC and HVA were only quantified in treated rats. Thus results were expressed as the total amounts per gram of wet tissue. 3-*O*-Medopa levels in treated rats were also expressed as ng g^{-1} of tissue for all structures.

In the striatum, DA, DOPAC and HVA contents increased significantly versus control during the first 0·5 h following L-dopa administration. In contrast, at the same time, 3-MT levels decreased (Fig. 1). The maximal increases in DA (+300%), DOPAC (+2700%) and HVA (+1700%) levels were obtained 1·5 h after treatment for the neurotransmitter and 1·5 or 4·5 h, respectively, for the metabolites. The lowest level of 3-MT (−40%) was observed after 1·5 h.

In the hypothalamus, L-dopa induced increases in DA and its three metabolite concentrations with a maximum level of DA (+1200%), DOPAC (+16000%), HVA (+4000%) and 3-MT (+60%) at times, 1, 1·5, 4·5 and 3 h, respectively (Fig. 1).

In the hippocampus, dopamine and its two major metabolites (DOPAC and HVA) were barely detectable in controls but were measurable 0·5 h after treatment. The maximal amount of DA (5·7 nmol g^{-1}), DOPAC (24·5 nmol g^{-1}) and HVA (16·2 nmol g^{-1}) were reached, respectively, 1·5, 3 and 3 h after L-dopa. These compounds fell to the detection limit 7·5 h after treatment (Fig. 2).

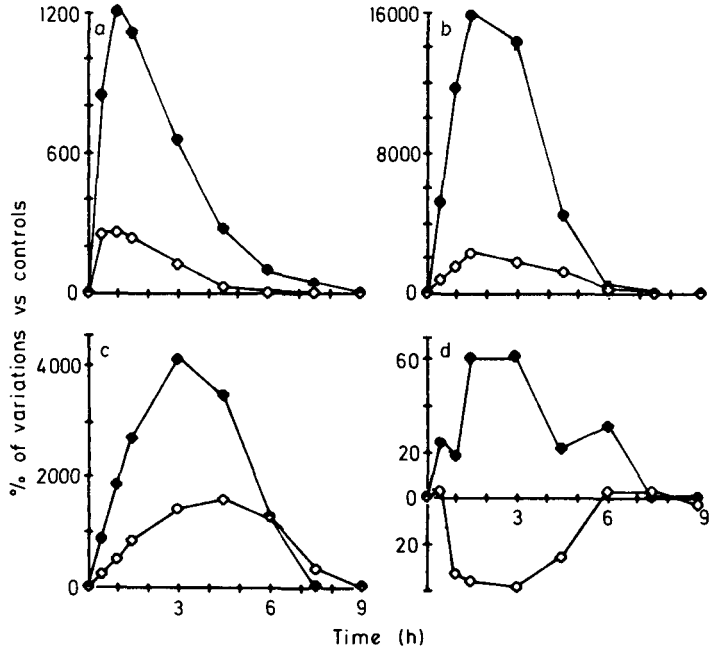

FIG. 1. Time course of (a) DA, (b) DOPAC, (c) HVA and (d) 3-MT levels in (●) hypothalamus and (○) striatum of rats treated with L-dopa + benzeraside. Results are expressed as percentage of variations versus control. Values are means of 5 determinations and standard deviations never exceeded ± 15%. L-Dopa (125 mg kg^{-1} i.p. route) was administered 0·5 h after benserazide (50 mg kg^{-1} i.p. route) and animals were killed from 0·5 to 9 h after L-dopa.

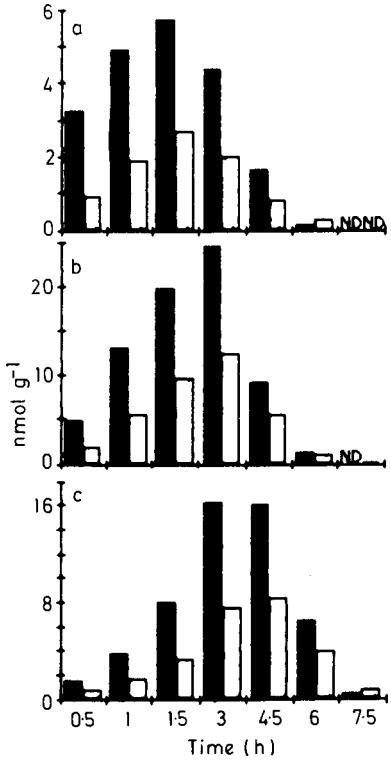

FIG. 2. Time course of (a) DA, (b) DOPAC and (c) HVA levels in the (■) hippocampus and (□) cerebellum of rats treated with L-dopa + benserazide. Values are expressed as nmol g^{-1} (wet tissue) and are means of 5 determinations. Standard deviations never exceeded ± 15%. L-Dopa (125 mg kg^{-1} i.p. route) was administered 0·5 h after benserazide (50 mg kg^{-1} i.p. route) and animals were killed from 0·5 to 9 h after L-dopa.

In the cerebellum of the treated rats, the maximal levels of DA (2·7 nmol g^{-1}), DOPAC (12·3 nmol g^{-1}) and HVA (8·3 nmol g^{-1}) were obtained at 1·5, 3 and 4·5 h after L-dopa loading. As in the hippocampus, the detection limit was reached 7·5 h after treatment (Fig. 2).

3-*O*-Medopa, was not detectable in control rats, whereas it appeared in all four brain areas of the treated rats. The maximal increase was observed at 4·5 or 6 h after L-dopa treatment, depending on the area. 9 h after administration, this derivative still persisted at high levels in hypothalamus, hippocampus and striatum (Fig. 3).

Discussion

After treatment by L-dopa and benserazide, the concentration of DA, DOPAC and HVA increased in the striatum, hypothalamus, hippocampus and cerebellum. However, the greatest intensity of this effect was in structures that were less dopaminergic. In the cerebellum and hippocampus these compounds, although not detectable in control rats, became measurable in treated animals and their levels rose from 2 to 16 times higher in the hypothalamus than in the striatum. The higher increase occurred with a time-lag between each metabolite according to the metabolic pathways, i.e. maximum DA levels appeared at first, followed by DOPAC peak concentrations, then by HVA maximal increases. This phenomenon was especially obvious in the striatum. Present data showed that the striatum, which is the main dopaminergic tissue, was able to exert a better regulation when the loading of the dopamine precursor was very high while the other structures were not able to overcome an excess of L-dopa. When considering a brain region such as cerebellum which is comparable to striatum with a complete lesion of its dopaminergic input, the increase in DA level could be related to a decarboxylation of L-dopa that occurs outside the dopaminergic pathway, as reported by Ng et al (1972) in-vitro and by Hefti et al (1981) in-vivo.

In addition to the main dopaminergic metabolites (DOPAC and HVA), we also measured a third metabolite of DA, namely 3-MT, in the hypothalamus and striatum. We found that 3-MT levels were increased in the hypothalamus but decreased in the striatum which is in apparent contradiction with the results of Ponzio et al (1984) who obtained an increase of striatal 3-MT in the rat, after oral treatment with L-dopa (100 mg kg^{-1}) plus benserazide (25 mg kg^{-1}). Those authors also reported an increase of DA by 27% in the striatum while we observed an increase by 300% for the same brain area. These discrepancies are likely to be due to a better bioavailability of L-dopa when given by the i.p. route. 3-MT decreased levels reflect a lower DA release in striatum (Di Giulio et al 1978) which can be related to the DA negative feedback regulation as described by Guiorguieff-Chesselet et al (1980). Such a phenomenon is not observed in the hypothalamus. Thus, data obtained with 3-MT substantiate the above hypothesis that striatum has a better regulation mechanism for restoring biochemical equilibrium after L-dopa loading.

Furthermore, 3-*O*-Medopa (a product of L-dopa methylation by COMT), which has been found previously in the striatum (El Gemayel et al 1986), also appeared in the other brain areas without a preferential distribution. Levels of this metabolite reached their highest values at nearly the same time whatever the brain structure. They remained very high 9 h after L-dopa loading. This slow disappearance is probably due to a weak cerebral clearance, in agreement with the hypothesis of Kuruma et al (1970). By these particular characteristics, 3-*O*-Medopa is quite different from the other metabolites normally found in the physiological conditions.

The comparative study of the dopaminergic metabolism in different rat brain areas after L-dopa loading as a pharmacological tool, provides further and direct evidence that the efficiency of the DA metabolism regulation is related to (dependent upon) the dopam-

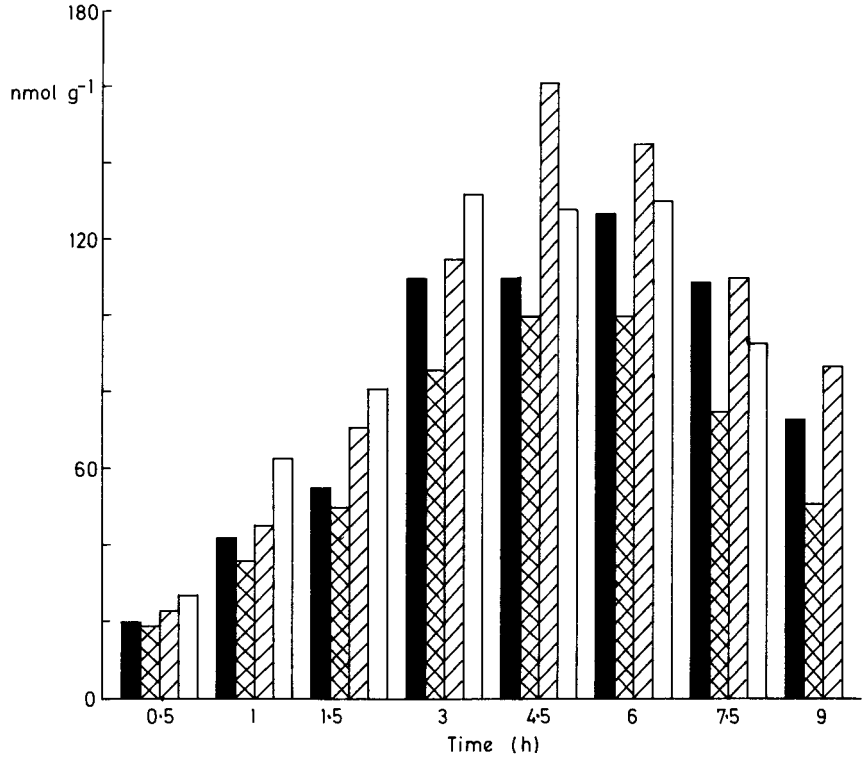

FIG. 3. Time course of 3-*O*-Medopa levels in the striatum, hypothalamus, hippocampus and cerebellum of rats treated with L-dopa + benserazide. Values are expressed as nmol g^{-1} (wet tissue) and are means of 5 determinations. Standard deviations never exceeded ± 15%. L-Dopa (125 mg kg^{-1} i.p. route) was administered 0·5 h after benserazide (50 mg kg^{-1} i.p. route) and animals were killed from 0·5 to 9 h after L-dopa. Key to columns: black, striatum; cross-hatched, hypothalamus; hatched, hippocampus; white, cerebellum.

inergic activity of the tissue. It seems that enzymatic activity, especially DOPA-decarboxylase and monoamine oxidase, allows the L-dopa loading in the striatum to be preferentially overcome while COMT, an extraneuronal and non-specific enzyme, equally transforms L-dopa into 3-*O*-Medopa in all the brain areas.

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