J. Pharm. Pharmac., 1974, **26**, 261–264 *Received November*, 5, 1973

Effect of L-dopa in ^{54}Mn incorporation by tissues

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^{54}Mn incorporation by tissues was studied in 24 rats fed with excess manganese diet and in 31 fed a normal diet. The effect of intraperitoneally administered L-dopa (100 mg kg^{-1}) on randomly selected rats in each group was determined. Manganese uptake in liver was higher in normally fed rats that received L-dopa; however, the manganese loaded rats showed a lower level of ^{54}Mn incorporation than those on a normal diet. No difference in ^{54}Mn incorporation was found in submaxillary glands from any of the groups studied. In brain tissue, 2 h after injection of L-dopa, the ^{54}Mn radioactivity was the same in normal and manganese-loaded animals and significantly higher than in rats not given the amine. The reverse effect was found in serum which from L-dopa-treated rats showed lower radioactivity than the controls, suggesting an increased passage of the tracer to the tissues. The different response of tissues from manganese-loaded rats could mean that the enzyme adenyl cyclase may be affected by the excess of manganese. Since dopamine does not cross the blood brain barrier, the L-dopa increase of ^{54}Mn uptake by brain could occur in the endothelial cells of the capillaries.

Mena, Court & others (1970) demonstrated the value of L-dopa in treating chronic manganese poisoning, but the metabolic changes that occur in such patients appear not to have been examined. Published work refers to the use of L-dopa in normal animals, but it is not known if L-dopa affects normal and manganese-loaded individuals similarly. Manganese dioxide intoxication in monkeys produces a decrease in striatal dopamine and 5-hydroxytryptamine concentrations (Neff, Barrett & Costa, 1969). Mustafa & Chandra (1971) showed that intratracheally administered manganese dioxide caused a lowering in the concentration of dopamine and adrenaline in the brain of rabbits, but 5-hydroxytryptamine was found to be unaltered. The authors have observed a decrease in dopamine and homovanillic acid content in rat brain after excess dietary manganese administration (Bonilla & Diez-Ewald, unpublished results). These findings suggest that chronic manganese poisoning effects are related to lowering of the catecholamines in the cerebrum. Papavasiliou, Miller & Cotzias (1968) demonstrated in rats that L-dopa caused diminished excretion of intravenously administered ^{54}Mn accompanied with its accumulation in the liver; these effects were possibly mediated by 3′5′-cyclic AMP. These reported observations led us to examine the effect of L-dopa on the incorporation of ^{54}Mn by liver, brain and submaxillary glands from normal and overloaded rats.

METHODS

Fifty-five Sprague-Dawley female rats, 200–300 g, had free access to Ratarina Laboratory Chow (Protinal Maracaibo, Venezuela). To 24 of these, 5 mg of $MnCl_2$

was added per ml of drinking water (distilled and demineralized) and the remaining 31 rats received similarly treated water without manganese. After 7 months on this diet, all the animals were injected intraperitoneally with 6μCi, of carrier-free ^{54}Mn Cl_2 (Amersham Searle, Illinois) dissolved in 0·5 ml of normal saline. Fourteen randomly selected rats from the manganese-loaded group, and 20 from the normal group, were simultaneously injected with L-dopa (100 mg kg^{-1}) suspended with continuous magnetic stirring in the same $^{54}MnCl_2$-normal saline solution. The remaining animals were used as controls. Thirty, 60 and 120 min after injection some animals were killed under ether anaesthesia, before the withdrawal of blood by cardiac puncture. Immediately after blood extraction, brain, livers and submaxillary glands were removed and stored frozen at −30°. After the removal of the organs from the peritoneal cavity, no remains of the L-dopa suspension were found. In all the animals injected with L-dopa, brain dopamine content was found to be higher than in rats not injected with the amine (Bonilla & Diez-Ewald, unpublished results).

Radioactivity was determined in a well-type, scintillation counter (Packard Instruments, La Grange, Illinois). The data were statistically evaluated utilizing the Student's *t*-test.

RESULTS

Liver. ^{54}Mn incorporation was higher in the L-dopa-treated animals fed on a normal diet than in their corresponding controls (Fig. 1). However, ^{54}Mn uptake by the manganese-loaded rats injected with L-dopa was lower than in the non-loaded rats not given the drug. There was no significant difference in the incorporation of ^{54}Mn between the two groups treated with a dietary excess of manganese. The non-loaded animals that were injected with L-dopa reached the highest uptake 60 min after injection, showing a significant statistical difference from their control ($P<0·02$). When the two L-dopa-treated groups (manganese-loaded and normal animals) were compared, we found a significant difference 60 min ($P<0·01$) and 120 min ($P<0·05$) after injections.

It is clear that L-dopa reached peak action 60 min after its intraperitoneal injection.

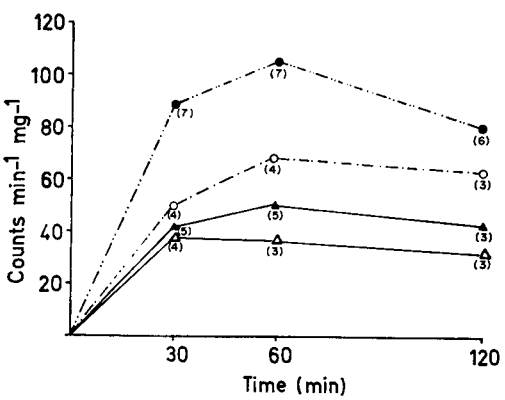

FIG. 1. Liver ^{54}Mn retention after injection of L-dopa. Figures in parentheses represent number of animals. ○, control; ●, control + L-dopa; △, manganese loaded; ▲, manganese loaded + L-dopa.

Submaxillary glands. In contrast to the findings in liver, we found that the submaxillary glands of manganese-loaded rats and their controls had a similar uptake of ^{54}Mn. There was also no significant difference between the L-dopa-treated groups and between these and the groups not treated with L-dopa.

Brain. Two h after L-dopa administration in normal rats, the radioactivity of brain remained high ($P<0{\cdot}02$) regardless of any previous diet. In contrast with liver, there was no difference between the ^{54}Mn uptake by the loaded and non-loaded groups after L-dopa administration (Fig. 2).

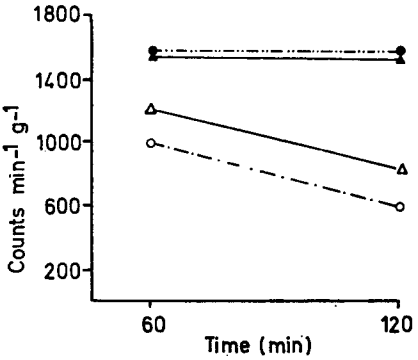

FIG. 2. Brain ^{54}Mn retention after injection of L-dopa. Symbols as in Fig. 1.

Serum. The L-dopa-treated groups showed effects in serum opposite to those seen in liver gland and brain (Fig. 3). ^{54}Mn radioactivity was lower in the L-dopa-treated animals than in their controls regardless of the previous manganese administration in the diet. These effects were more pronounced 30 min after the L-dopa injections ($P<0{\cdot}001$); however, 60 min later, we did not find any difference between the control groups (with and without L-dopa), although the difference persisted for the loaded animals ($P<0{\cdot}001$). In the groups not injected with L-dopa the manganese-loaded rats showed a higher radioactivity than the controls 60 min after injection of the tracer ($P<0{\cdot}001$).

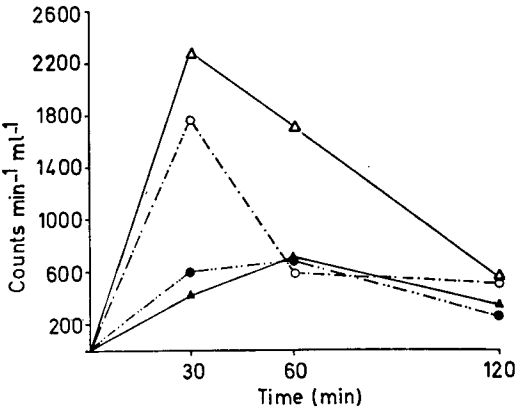

FIG. 3. Serum ^{54}Mn retention after L-dopa injection. Symbols as in Fig. 1.

DISCUSSION

Our results agree with those reported by Papavasiliou & others (1968) in liver of normal rats. However, the increased uptake of ^{54}Mn after L-dopa administration seems to be a phenomenon that occurs in other organs, i.e. the brain. In serum, the low radioactivity after L-dopa administration most probably indicates an increased passage of the tracer to the tissues. The enhancing effect of L-dopa on ^{54}Mn uptake by liver and brain was also found in rats subjected to an excessive manganese intake. Since this process has been suggested to be mediated through an activation of adenyl cyclase (Papavasiliou & others, 1968), the findings reported here suggest that the enzyme could be affected by the excess of manganese in tissues. Despite the fact that liver from loaded rats increases ^{54}Mn uptake after L-dopa, this was at a lower level than in normal animals not injected with L-dopa. This suggests that uptake of manganese by the liver could inhibit the adenyl cyclase system. We have previously shown that manganese load does not affect ^{54}Mn incorporation by submaxillary glands (Bonilla & Diez-Ewald, 1972). According to the present findings, L-dopa tends to increase the uptake of isotope. In brain, the rate of ^{54}Mn radioactivity after L-dopa was similar for both manganese-loaded and normal rats, in spite of the 7 months of dietary manganese excess. In monkeys, in which intraperitoneal injections of manganese chloride during 10 to 20 months caused an increased manganese concentration in liver, reaching values more than 40 times higher than those from normal animals, Mella (1924) found, in contrast, that brain concentrations only increased 5–7 times. These findings and our results seem to indicate that brain never reaches the high manganese concentration of liver, probably because of the restrictions imposed by the blood-brain barrier. There is another important fact to be considered. That is that brain adenyl cylase has an activity (protein basis) 22 times higher than in liver and its concentration in units per 100 g (wet weight) is about 8 times that of liver (Sutherland, Rall & Menon, 1962). So, in spite of the increased manganese content in brain, the concentration reached could not be high enough to inhibit the total enzyme present.

Most of the acutely administered L-dopa is transformed into dopamine in extracerebral tissues and at the endothelial lining of brain capillaries (de la Torre & Mullan, 1972). Since dopamine does not cross the blood-brain barrier (Bertler, Falck & Rosengren, 1963) the L-dopa increase of ^{54}Mn uptake by brain could occur neither in the neurons nor in the glia, but rather in the endothelial cells of the capillaries.

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