# L-Dopa-Induced Release of Cerebral Monoamines

Abstract. *L-Dopa markedly increased the efflux of tritiated dopamine and tritiated serotonin from rat brain slices. This action appeared contingent on the decarboxylation of* L-*dopa to dopamine, since it could be blocked by an inhibitor of* L-*amino acid decarboxylase. Selective destruction of catecholamine-containing nerve terminals by 6-hydroxydopamine significantly decreased the uptake and release of tritiated dopamine but not that of tritiated serotonin. These observations support the hypothesis that a portion of exogenously administered* L-*dopa may enter central serotonin terminals and undergo decarboxylation to the amine with resultant displacement of the endogenous indoleamine from vesicular stores.*

Although the efficacy of L-dopa (L-dihydroxyphenylalanine) in the symptomatic treatment of parkinsonism is now well established, relatively little is known of the drug's central pharmacologic actions. Recent studies indicate that in addition to elevating brain dopamine (DA) concentrations, large doses of L-dopa substantially reduce central serotonin stores, while initially increasing concentrations of the principal metabolite of serotonin, 5-hydroxyindoleacetic acid (*1*). Histochemical observations suggest that DA formed from exogenous L-dopa can accumulate in serotonergic as well as dopaminergic neurons (*2*). These findings support the hypothesis that a portion of exogenously administered L-dopa may enter central serotonin terminals, undergo decarboxylation to DA, and then displace the endogenous indoleamine from vesicular stores. Here we report observations in vitro which support this contention and which may have relevance to the mechanisms of action of L-dopa in the treatment of parkinsonism.

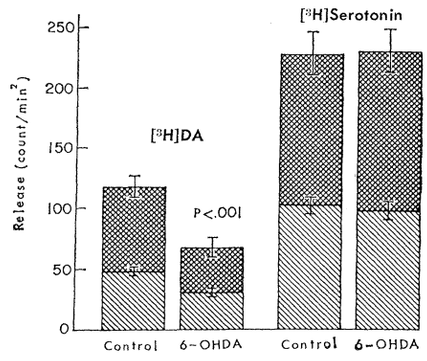


Fig. 1. Effect of prior treatment with 6-hydroxydopamine (6-OHDA) on spontaneous and L-dopa-induced efflux of radioactivity from rat brain slices previously incubated with [³H]DA or [³H]serotinin. Lined areas of the bars indicate spontaneous tritium efflux before L-dopa, and the total height of each bar depicts the maximum efflux during the first 6 to 12 minutes of drug exposure; the cross-hatched areas represent release induced by $1 \times 10^{-4}M$ L-dopa. Each result is the mean (± S.E.M.) for six or more slices of frontal cortex.

Adult Sprague-Dawley rats were decapitated and slices of frontal cortex and corpus striatum weighing about 20 mg were rapidly prepared (*3*). Tissues were incubated at 37°C in a supplemented Krebs-Ringer medium, saturated with 5 percent $CO_2$ in $O_2$ and containing either [³H]DA (16 c/mmole; 10 ng/ml) or [³H]serotonin (6 c/mmole; 15 ng/ml). After the slices were incubated for 30 minutes, they were transferred to individual chambers through which fresh oxygenated medium was rapidly circulated. After 40 minutes of superfusion, when the efflux of radioactivity had fallen to a nearly steady level, the superfusing medium was switched to one containing L-dopa. Sequential samples of effluent superfusate, collected at 2-minute intervals before and during drug exposure, were analyzed for total tritium by liquid scintillation spectrometry. The results are expressed as the average tritium efflux during the 6-minute interval just prior to drug exposure and maximum efflux during the interval from 6 to 12 minutes after beginning drug exposure.

L-Dopa produced a substantial increase in the tritium efflux from striatal slices previously incubated with [³H]DA or [³H]serotonin (Table 1). Analysis by column chromatography (Dowex-H+) (*4*) showed that the tritium efflux induced by L-dopa consisted predominantly of the unmetabolized amine. The release of [³H]serotonin induced by L-dopa occurred not only from striatal tissues, which are rich in DA terminals, but also from frontal cortex (Fig. 1) where such terminals are relatively scarce, suggesting the participation of norepinephrine or serotonin neurons. The presence of an L-amino acid decarboxylase inhibitor, α-methyldopa-hydrazine (MK 485) (*5*), in the superfusing medium completely blocked the release of [³H]DA or [³H]serotonin induced by L-dopa (Table 1). The MK 485 alone at the concentration used did not significantly influence the uptake of L-dopa or alter the spontaneous release of either labeled amine. Since L-dopa is largely decarboxylated to DA in brain, it is likely that the release of serotonin induced by L-dopa is mediated by DA, possibly through displacement of serotonin from vesicular stores. This possibility is supported by the finding that DA ($1 \times 10^{-5}M$) itself is capable of releasing [³H]serotonin from rat brain slices and that this action is not affected by MK 485 (*6*).

In other experiments, rats were given prior treatment with 6-hydroxydopamine to destroy catecholaminergic nerve terminals (*7*). Under light ether anesthesia, the animals received three doses of 200 μg of 6-hydroxydopamine hydrobromide by intracisternal injection at intervals of 48 hours. The control rats were injected with an equal volume (20 μl) of vehicle solution (normal saline containing 0.01 percent ascorbic acid, adjusted to *p*H 4.3). All animals were killed 10 to 30 days after the final injection. Analysis of endogenous amines (*8*) in whole brains of rats treated with 6-hydroxydopamine showed a depletion of norepinephrine and DA to 16 ± 2.2 and 32 ± 4.9 percent, respectively, of control values; concentrations of serotonin in the brain were not significantly altered. Prior treatment with 6-hydroxydopamine reduced significantly the uptake of [³H]DA into frontal cortex slices

Table 1. Effect of L-dopa on the efflux of radioactivity from rat striatal slices previously incubated with [³H]dopamine or [³H]serotonin. After 40 minutes of superfusion with or without added MK 485 ($3 \times 10^{-4}M$), the medium was switched to one containing L-dopa ($1 \times 10^{-5}M$). The results are expressed as counts per minute per minute (means ± S.E.M.) obtained in four or more slices.

| Drug added | Release | | |
|---|---|---|---|
| | Before L-dopa | During L-dopa | L-Dopa induced |
| | *[³H]Dopamine* | | |
| None | 91 ± 8 | 225 ± 24 | 135 ± 18* |
| MK 485 | 80 ± 2 | 76 ± 3 | −4 ± 3 |
| | *[³H]Serotonin* | | |
| None | 98 ± 4 | 204 ± 16 | 106 ± 11* |
| MK 485 | 124 ± 8 | 125 ± 9 | 1 ± 5 |

* $P < .01$.

to 43 ± 2.2 percent of control values. The uptake of [$^3$H]serotonin was not significantly altered (98 ± 6.3 percent of controls). Both spontaneous and L-dopa-induced release of [$^3$H]DA was significantly diminished by prior treatment with 6-hydroxydopamine, while the release of [$^3$H]serotonin remained unchanged (Fig. 1).

The foregoing observations provide direct evidence that L-dopa releases brain DA and serotonin and that this releasing action may be dependent on the conversion of dopa to DA. The effect of L-dopa appears to be specific, since it did not influence the release of [$^{14}$C]urea from striatal slices (*9*). Furthermore, selective destruction of catecholamine-containing nerve terminals by 6-hydroxydopamine substantially decreased the uptake and release of [$^3$H]DA but not that of [$^3$H]serotonin. Although it is likely that some decarboxylation of L-dopa to DA continues in catecholaminergic terminals remaining after prior treatment with 6-hydroxydopamine, it is improbable that the amine thus formed could account entirely for the unaltered release of [$^3$H]-serotonin. Our results thus suggest that the presence of catecholaminergic terminals are not necessary for the L-dopa-induced release of serotonin and that the uptake and decarboxylation of L-dopa to DA occur within serotoninergic as well as dopaminergic neurons.

These findings may have important clinical implications. Conceivably L-dopa when forming abnormally large concentrations of dopamine may interfere with the metabolism of some other essential amine or displace it from its normal storage sites. In patients treated with high doses of L-dopa a decrease of serotonin resulting from displacement by DA seems therefore highly probable. The role of DA as a central "false" serotoninergic transmitter may be relevant to the therapeutic effects or side effects, or both, observed in patients receiving high doses of L-dopa.

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## Intestinal Hydrolysis and Conjugation of a Pesticidal Carbamate in vitro

Abstract. *The metabolite 1-[1-$^{14}$C]naphthyl glucuronide was isolated from mucosal and serosal fluids of everted sacs of rat small intestine incubated in media containing either the insecticide 1-[1-$^{14}$C]naphthyl N-methylcarbamate (carbaryl) or 1-[1-$^{14}$C]naphthol. The hydrolysis of carbaryl and conjugation of the liberated naphthol indicated some degree of metabolism by the intestine before absorption.*

Conjugation of one chemical with another is a common metabolic function in mammals. Conjugates of insoluble toxic chemicals are generally more water-soluble and less toxic than the parent chemical (*1*).

Although the liver has a high capacity to perform the metabolic process of conjugation, it is important to recognize that other tissues also have this capability. Metabolic conjugation occurs in the kidney, gastric mucosa (*2*), and intestine (*3*).

Pesticide chemicals, ingested as residues on foodstuffs, encounter the gastrointestinal tract before any other tissue. If these pesticides were conjugated by the intestine, their solubility, and consequently their absorption and toxicity, would be altered. Reduced absorption or toxicity or both would provide substantial protection against the chemical.

Sulfate and glucuronide conjugates of various carbamate pesticidal chemicals or their hydrolysis products or both were reported to have been found in urine (*4–6*). The 1-[1-$^{14}$C]naphthyl glucuronide conjugate was among the major polar metabolites of 1-[1-$^{14}$C]-naphthyl *N*-methylcarbamate in rat and guinea pig urine (*4*), and to a lesser extent in chicken urine (*6*). Since naphthyl glucuronide represents one of the major polar urinary metabolites of the rat and methods for its isolation from biological samples have been described (*6*), it was selected as a model compound to investigate the ability of rat intestine to synthesize this specific glucuronide from carbaryl.

The small intestines of male Sprague-Dawley rats (275 to 350 g) were divided into three approximately equal parts and everted by use of the general technique of Wilson and Wiseman (*7*). The serosal space of the sacs was filled with 4 ml of the appropriate Krebs-Ringer medium (*8*), containing 1 mg of glucose per milliliter, and the sacs were individually incubated for 2 hours at 37°C in 100 ml of the same medium (mucosal fluid) under an atmosphere of 95 percent $O_2$ and 5 percent $CO_2$. After incubation, the serosal and mucosal fluids from all sacs

Table 1. The percentage of the total $^{14}$C in that portion of mucosal and serosal fluids which partitioned into the aqueous phase, and the *minimum* percentage of the carbon-14 in the aqueous phase that was accounted for as 1-[1-$^{14}$C]-naphthyl glucuronide (NG). The minimum percentage is the ratio of the disintegrations per minute of final isolated metabolite to the disintegrations per minute in the original aqueous phase after partition times 100.

| Substrate | Fluid | Aqueous phase $^{14}$C | |
|---|---|---|---|
| | | Total (%) | As NG (minimum %) |
| *Krebs-Ringer bicarbonate medium* (pH 7.4) | | | |
| 1-[1-$^{14}$C]naphthyl *N*-methylcarbamate | Mucosal | 21 | * |
| | Serosal | 68 | 67 |
| *Krebs-Ringer phosphate medium* (pH 6.5) | | | |
| 1-[1-$^{14}$C]naphthyl *N*-methylcarbamate | Serosal | 40 | 46 |
| *Krebs-Ringer bicarbonate medium* (pH 7.4) | | | |
| 1-[1-$^{14}$C]naphthol | Mucosal | 91 | 35 |
| | Serosal | 99 | 58 |

* Insufficient quantity of compound for analysis.