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Time and Dose Influences on the Behavioral Effects of L-DOPA and 5-Hydroxytryptophan after Inhibition of Extracerebral Decarboxylase[1,2]

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GRONAN, R. J. *Time and dose influences on the behavioral effects of L-DOPA and 5-hydroxytryptophan after inhibition of extracerebral decarboxylase.* PHARMAC. BIOCHEM. BEHAV. 3(2) 161–166, 1975. – The influence of time and dose factors on the locomotor activity and gross behavioral effects of L-3,4-dihydroxyphenylalanine (L-DOPA) and 5-hydroxytryptophan (5-HTP) were assessed in albino mice pretreated with the peripheral decarboxylase inhibitor, MK-486. L-DOPA caused stereotyped behaviors and a decrease in orienting responses and locomotor activity, followed at the highest dose by increased locomotor activity. 5-HTP caused a dose-dependent increase in orienting responses and locomotor activity followed after 90 min by a dose-dependent decrease in locomotor activity. Administration of MK-486 and L-DOPA followed in the same animal by 5-HTP resulted in a diminution of the effects seen after either drug alone. This apparent antagonism may result from competition between the two amino acids at sites of membrane transport in the brain.

L-DOPA 5-Hydroxytryptophan Locomotor activity Behavior

THE administration of L-dihydroxyphenylalanine (L-DOPA) produces an increase in the amount of its catecholamine metabolite dopamine (DA) in both the central nervous system (CNS) and various peripheral tissues [8]. Similarly, administration of 5-hydroxytryptophan (5-HTP) causes a rise in tissue levels of its decarboxylated metabolite, 5-hydroxytryptamine (5-HT) [27]. The decarboxylation of either of these amino acid precursors can be inhibited in peripheral tissues by prior administration of an inhibitor of their common decarboxylating enzyme (e.g., α-hydrazino-α-methyl-β-(3,4 dihydroxyphenyl) propionic acid: MK-485) which does not itself cross the blood brain barrier into the CNS [22]. The behavioral changes observed after this treatment are thought to be mediated primarily by the catecholamines or 5-HT thus formed in the brain.

One of the most consistently noted behavioral effects of L-DOPA in rodents is an alteration of locomotor activity. Some reports indicate that this effect of L-DOPA, given alone, may be dose dependent, in that doses up to 400 mg/kg decrease activity while high doses (500 mg/kg or more) cause increased locomotor activity [4, 6, 25]. Strömberg [25] demonstrated that pretreatment with MK-485 does not abolish the biphasic nature of this effect, and thus, that both the depressant and stimulatory actions are dependent on central mechanisms.

In an apparent conflict with these findings, however, doses of L-DOPA, that should thereby decrease activity, have been reported by other investigators to produce increased locomotor activity in mice and rats when administered after inhibition of extracerebral decarboxylase by either MK-485 (17) or RO-4-4602 (seryl-trihydroxybenzylhydrazine) [7,23].

A similar uncertainty surrounds the behavioral effects of 5-HTP. Given alone, 5-HTP has been shown to decrease activity in a variety of species [18, 19, 24, 26], although very high doses have been reported to cause increased locomotor activity and tremors in rats [5,14] and in mice pretreated with iproniazid [3,11]. After MK-486, the more potent L-isomer of MK-485, on the other hand, Modigh [19] measured a dose-dependent increase in locomotor activity in mice given 5-HTP. Other workers, however, have observed little or no behavioral change in rats given 5-HTP after administration of MK-486 [14] or RO-4-4602 [7].

The variety of behavioral effects reported to follow administration of L-DOPA or 5-HTP has somewhat hindered an understanding of the functional roles of their

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metabolites in the CNS. A close look at these studies suggests that some of their differences may be due to the different decarboxylase inhibitors or doses used, or to differences in the length of the period of observation of the animal's behavior. In the present investigation the latter two of these factors were assessed by measuring the time course of L-DOPA and 5-HTP-induced changes in mouse locomotor activity, after inhibition of peripheral decarboxylase by MK-486 [21]. Additional measures of interactions between the two amino acids were also taken. Three separate but similar experiments, designated 1, 2 and 3, were thus performed. In Experiment 1, saline or one of four doses of L-DOPA was given to MK-486 pretreated mice. In Experiment 2, saline or one of four doses of 5-HTP was administered to MK-486 pretreated mice. In Experiment 3 the activity of MK-486 pretreated mice was measured after sequential injections of both L-DOPA and 5-HTP.

METHOD

Animals

Fifty naive, adult male CF-1 (albino) mice weighing 30–40 g were used in each experiment. The mice were housed 5 to a cage under conditions of a 12:12, light–dark schedule for at least 3 weeks prior to use. Those in Experiments 1 and 2 were 80–100 days old, while those in Experiment 3 were 115–130 days old at time of testing.

Apparatus

Locomotor activity was measured by means of the "motimeter" described by Knoll [16]. In this device the animal moves over four aluminum contact plates mounted 4 mm apart in a clear Plexiglas box (testing cage) and a count is recorded for every passage between two plates. Each mouse was tested singly in 1 of 5 identical testing cages and observation of gross activity was made simultaneously with the automatic recording of locomotor activity.

Drugs

Crystalline L-β-3,4-dihydroxyphenylalanine (L-DOPA) and 5-hydroxy-DL-tryptophan (5-HTP) were supplied by Sigma Chemical Company. L-α-hydrazino-α-methyl-β-(3,4-dihydroxyphenyl) propionic acid (MK-486) was kindly donated by Dr. Clement A. Stone of the Merck Institute for Therapeutic Research. 5-HTP was dissolved in sterile saline (0.9% NaCl, pH 5.5) with warming. L-DOPA and MK-486 were dissolved in 0.1 N HCl/saline with warming and the pH was adjusted to 5.5 with $NaHCO_3$/saline solution. Control injections were 0.9% saline. All drug solutions were prepared immediately before injection and were given intraperitoneally.

Procedure

Each of the mice in a particular housing cage was randomly assigned to 1 of the 5 treatment levels and placed alone in a testing cage at the start of the dark period of the light-dark cycle. Continuous recording of locomotor activity was begun at this time. After allowing 30 min for habituation to the apparatus, all mice were given MK-486 (100 mg/kg, I.P.), and after an additional 30 min, they received L-DOPA or 5-HTP (or saline) in Experiments 1 and 2 respectively. Systematic observation of the animal's gross behavior was begun at this time, with particular reference to some effects previously recorded for these drugs: stereotyped hyperkinesis [7], irritability and jumping [6,11] after L-DOPA, and "head-twitches" [9] and excitability [3] following 5-HTP. MK-486 was injected at a concentration of 6.0 mg/ml. L-DOPA and 5-HTP were injected at concentrations up to 12.5 and 15.0 mg/ml, respectively, which are saturated solutions at pH 5.5. Thus, a large injection volume (0.04 ml/g of mouse) was required to keep the high dose of each of these drugs in solution, and this volume was made the same for all doses and the MK-486/saline controls. In Experiment 3, MK-486 was similarly given to all animals at 30 min after entry into the cage, L-DOPA or saline at 60 min (0.04 ml/g of mouse), and 5-HTP or saline at 90 min (0.02 ml/g of mouse). The L-DOPA and 5-HTP were given in this sequence to the two groups receiving them for three reasons: it was desirable to keep the injection volume at any one time as low as possible and to minimize competition between the amino acids for entry into the CNS and finally, the earlier studies showed that this would cause their peak excitatory effects to approximately coincide in time. The doses used in each experiment are shown in Figs. 1–3.

RESULTS

Experiment 1

The animals receiving L-DOPA in Experiment 1 displayed a variety of characteristic dose-dependent behaviors, which are reflected in the graphs of their activity scores in Fig. 1. Intermediate doses decreased activity while the highest dose (500 mg/kg) caused increased irritability and locomotor activity. The depressant effect, seen most strongly in the 125 mg/kg group, was characterized by stuporous immobility, marked ptosis or eye squint and muscular flaccidity for approximately 15–120 min after L-DOPA administration. The behavior of the 250 mg/kg group was similar but also included a Straub tail-like response and a very prominent stereotyped sniffing and stretching of the neck, interrupted intermittently by brief running or jumping movements. These animals were highly irritable but oriented very poorly to irritating stimuli, squealing and jerking away, or assuming defensive postures when touched, and biting when held. The animals given the highest dose of L-DOPA progressed sequentially through the syndromes just described for the lower doses, during the first 30 min after drug injection. The running episodes thereafter became more frequent, until by one hour after L-DOPA, the mice showed a repetitive pacing of the length of the testing cage and stereotyped movements of the head.

Differences among dose effects at the two one hour time periods (indicated as a and b in Fig. 1) were tested by a two-way analysis of variance for repeated measures within drug doses, using logarithmically transformed raw scores [15]. This transformation was necessary since the raw scores did not meet the requirement of homogeneity of variance, according to the F_{max} test of Hartley [13]. The computed F values for Dose effects, Time effects, and Interaction (Dose × Time) were all significant beyond the 0.01 level of probability. This analysis was followed by Tukey's *a posteriori* test of differences between selected pairs of means [15]; significant differences are indicated in Fig. 1.

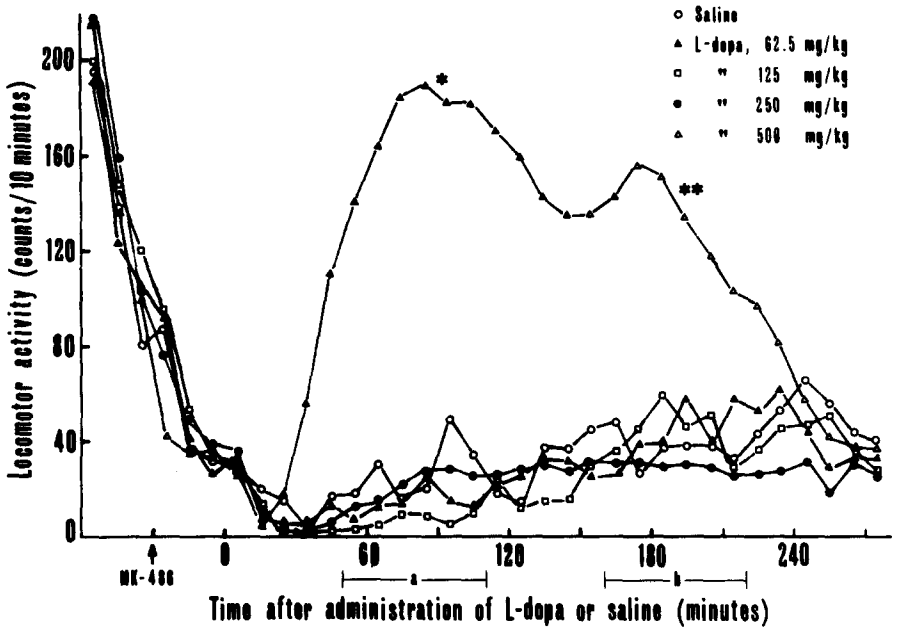


FIG. 1. Mean locomotor activity (counts/10 min) in mice after MK-486 and various doses of L-DOPA, or saline, as a function of time. Counts are shown at the midpoint of each 10 min period for which they were recorded. * Significantly different from all other groups during Period a ($p<0.01$). ** Significantly different from control during Period b ($p<0.03$). There is a significant difference between scores of Periods a and b for the 125 mg/kg group ($p<0.01$).

Experiment 2

The behavioral effects observed after administration of different doses of 5-HTP generally differed only in their degree of intensity at any particular time, but there was a marked change in some of these effects over time, as may be seen in the locomotor activity curves of Fig. 2. During the first hour after injection of 5-HTP there was a dose-dependent increase in the general activity level of the mice. At the highest dose (600 mg/kg) this included very active exploration of the cage alternated with short periods of intensive preening and grooming, and frequent twitching of the head. These animals were very alert and quick to orient to any movements or sounds external to the testing cage. Their head movements were of two types, a frequent, quick visual reorientation involving the entire head, and a quick rotation (twitch) of the head around the longitudinal axis of the body, occurring every 5 to 15 sec at the highest dose. All of these behaviors were seen proportionally less frequently in the animals administered the three lesser doses of 5-HTP.

Approximately 80 min after 5-HTP there was a marked reversal of the exploratory excitation. The highest dose animals showed progressively more frequent periods of quiet sitting, although they remained alert to outside stimuli, and still showed an occasional head twitch. The lower dose groups showed a proportionately smaller reduction of locomotor activity during this period, as may be seen in Fig. 2.

An analysis of variance was performed as in Experiment 1, using untransformed scores. The two, one hour time periods chosen for this analysis were 30 min earlier than those used in Experiment 1, since these periods seemed to more accurately reflect the effects described for 5-HTP. The F values for Time and Interaction (Dose x Time) effects were significant beyond the 0.01 level while that for Dose effects did not reach this level of significance. Significant pairwise comparisons by Tukey's test are indicated in Fig. 2.

Experiment 3

The behavior of the control, L-DOPA (500 mg/kg) and 5-HTP (300 mg/kg) groups in this experiment was similar to that observed previously, as may be seen in Fig. 3. A dose of 125 mg/kg of L-DOPA given to the 5-HTP animals soon after that group's peak locomotor activity, did not significantly alter the subsequent locomotor depression when compared to the 300 mg/kg 5-HTP group in Experiment 2. The group administered both L-DOPA (500 mg/kg) and 5-HTP (300 mg/kg) (HD group) displayed a behavioral syndrome containing elements of both drug effects, although the stereotyped behavior seen after L-DOPA alone seemed to predominate in these animals. An analysis of variance of the logarithmically transformed activity scores recorded from these four groups during the 30 min period indicated as d in Fig. 3 revealed a significant Interaction effect ($p<0.01$). Significant pairwise comparisons by Tukey's test are also shown in Fig. 3.

While the activity of the L-DOPA and HD groups did not differ significantly during this initial period of analysis,

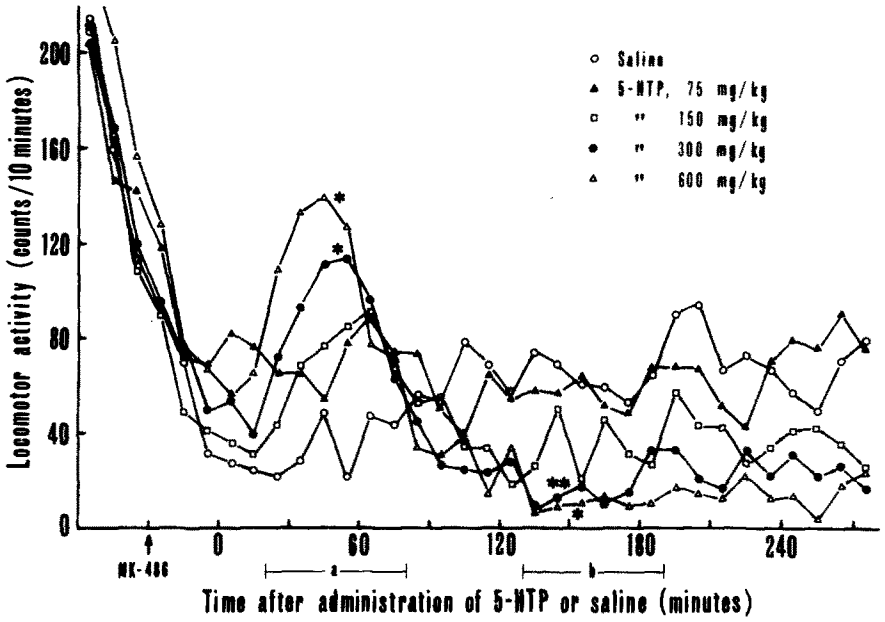
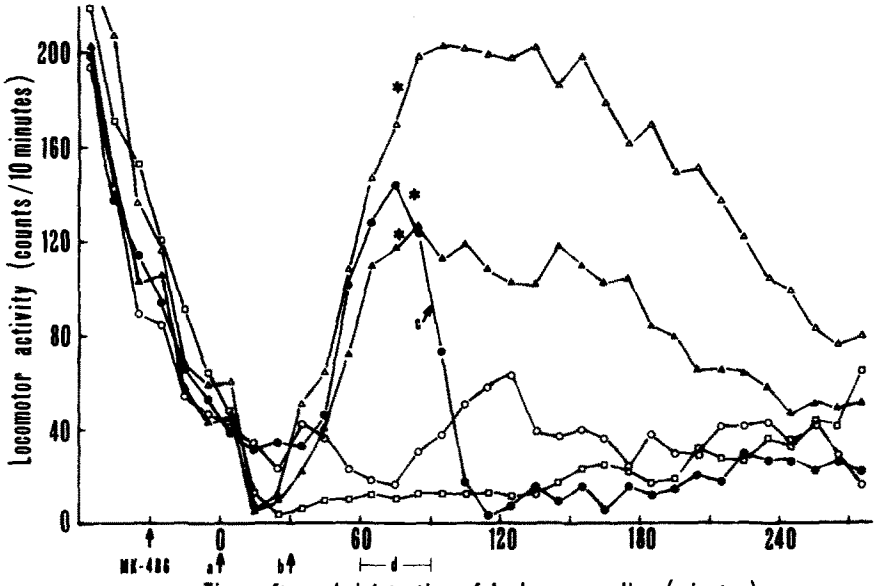


FIG. 2. Mean locomotor activity in mice after MK-486 and various doses of 5-HTP, or saline, as a function of time. * Significantly different from control during Period a or b ($p<0.01$). ** Significantly different from control during Period b ($p<0.03$). There is a significant difference between scores of periods a and b for each of the following groups: 600 mg/kg ($p<0.01$), 300 mg/kg ($p<0.01$), 150 mg/kg ($p<0.03$).



	Treatment Name	Time of Injection a	b	c
○	Control	Saline	Saline	—
△	L-dopa	L-dopa, 500 mg/kg	Saline	—
●	5-HTP	Saline	5-HTP, 300 mg/kg	L-dopa, 125 mg/kg
▲	HD	L-dopa, 500 mg/kg	" "	—
○	LD	L-dopa, 125 mg/kg	" "	—

FIG. 3. Mean locomotor activity in mice after MK-486 and saline, L-DOPA, and/or 5-HTP, as a function of time. * Significantly different from control during Period d ($p<0.01$). There is a significant difference between the 5-HTP and LD groups during Period d($p<0.001$) and between the L-DOPA and HD groups for the period 90–170 min after injection of L-DOPA ($p = 0.01$).

there was a significant difference in their scores (t test, p = 0.01) during a subsequent period (90–170 min after L-DOPA), indicating an attenuation by 5-HTP of the L-DOPA induced hyperactivity.

Those animals administered 125 mg/kg of L-DOPA (LD group) showed the depressed behavior described earlier for this dose. The subsequent administration of 5-HTP had little noticeable effect , and did not produce the excitation elicited by this dose of 5-HTP not preceded by L-DOPA. The difference between these two groups was found to be highly significant during the 30 min period (d) indicated in Fig. 3 (t test, $p<0.001$).

DISCUSSION

The dose-response relationships observed here for MK-486 and L-DOPA (Experiment 1) confirm the biphasic dose effects on locomotion observed by Strömberg [25] 70 min after treatment with this drug combination. It should be noted, however, that the initial effect of all doses tested was a reduction of locomotion and orienting responses; the increased locomotor activity subsequently measured at the highest dose was not accompanied by the normal exploratory movements and orienting responses shown by control animals. The hyperexcitability and spontaneous jumping reported previoulsy [6] for high doses of L-DOPA alone, was also observed in this study, primarily at the 250 mg/kg dose. In addition to these behaviors, the most obvious manifestation of a high dose of L-DOPA was the stereotyped sniffing and neck stretching. Similar effects have been reported by other investigators [7,25].

While the qualitatively different dose effects of L-DOPA as well as the time course of these effects shown in this study are consistent with a number of previous reports, these results are not in accordance with the increased locomotor activity reported previously for intermediate doses of L-DOPA following RO-4-4602 [7,23]; this may be due to differential effectiveness of the decarboxylase inhibitors or to differences in the strain and age of the experimental animals used. In addition, although L-DOPA has been observed to induce fighting in mice [6], such behavior was not easily manifested in this study because the mice were always tested in isolation.

The behavioral effects observed in Experiment 2 after administration of MK-486 and 5-HTP confirm those reported by Modigh [19] and indicate that a primary central effect of 5-HTP under these conditions is increased locomotor activity and an increase in orienting responses. The prolonged dose-dependent decrease in locomotor activity following this initial period has not previously been reported, however.

While high doses of both L-DOPA and 5-HTP have thus been shown to have reciprocal biphasic effects on mouse locomotor activity when administered after MK-486, it must be recognized that, taken alone, this measure can be a somewhat deceptive indicator of general behavior. By gross observation, the effects of these drugs were clearly different. L-DOPA decreased orienting responses and specific exploratory movements at all doses and this continued through the period of increased locomotor activity subsequently measured at the highest dose. 5-HTP, on the other hand, initially augmented visual and postural orienting responses and mice receiving this drug remained quite alert during the subsequent period of locomotor depression. While the stimulatory action of L-DOPA therefore appears to be specific for locomotor activity, the initial stimulatory effects of 5-HTP are more general, and the animal's behavior more closely resembles the normal excited state of untreated mice when first placed in the unfamiliar testing cage.

The finding that certain doses of L-DOPA and 5-HTP elicited a reciprocal biphasic alteration of mouse locomotor activity was especially interesting in light of alterations in brain monoamines that have been reported to occur after these drugs. In addition to increasing DA, high doses of L-DOPA have been found to elicit an increase in release and metabolism of brain 5-HT, apparently as a result of its displacement by DA [2,10]. Conversely, 5-HTP has been reported to cause an increase in 5-HT and a decrease in DA levels by an analogous process of displacement by 5-HT [7, 12, 20], suggesting the possibility that functional activity of the displaced amines may contribute to the behavioral syndromes observed.

In accordance with this idea, the increased locomotor activity seen in these studies as a secondary effect of a high dose of L-DOPA might be hypothesized to share a common basis (e.g., 5-HT release) with the increased activity observed as the primary effect of 5-HTP, and the injection of both drugs in a sequence which would allow these effects to coincide in time might be expected to cause an additive increase in locomotor activity.

This was not observed, however, in the HD group of Experiment 3, which did not show as high a level of activity as either of the groups given only L-DOPA or 5-HTP. This apparent antagonism may be the result of competition between the two amino acids, either for transport into the brain or at some intracerebral site, since high concentrations of L-DOPA remain in both blood and brain 30 min after its intraperitoneal administration [1], the time at which 5-HTP was given in this study. It should be noted, however, that mechanisms other than competition may be responsible for this effect. Nevertheless, the lower activity of the HD group would appear to support the view that these drugs increase locomotor activity in mice by different mechanisms in the brain.

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