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Effects of Alpha-methyldihydroxyphenylalanine, Reserpine and Dihydroxyphenylalanine on Pressor Responses to Norepinephrine and Tyramine in Humans.* (29479)

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In recent years the biogenic amines have become a subject of interest in the biological study of mental illness. The clinical relevance of this interest has increased with the availability of substances which affect the synthesis, storage, release, and degradation of these amines. Within this context we have been particularly interested in the question whether psychiatric patients in any of the various diagnostic categories show differential responses to such substances. Preliminary to such work it appeared important to ascertain the effects of several of these substances on selected normal subjects. This paper reports the effects of intravenous tyramine and norepinephrine on arterial systolic blood pressure in 4 subjects pretreated with alpha methyl-dihydroxyphenylalanine (alpha methyl DOPA), reserpine, dihydroxyphenylalanine (DOPA), and under control conditions. A peripheral measure—blood pressure—was chosen as a dependent variable not because it necessarily reflects central events but because it is available for study and is easily objectified.

Alpha methyldopa was of particular interest because of previous observations which showed it to have a paradoxical enhancing effect on tyramine infusion in hypertensive subjects(1). It was also of particular interest to attempt to repeat in humans, in relatively minute doses, the earlier observation of Burn and Rand(2) that the pressor effect of norepinephrine is increased by reserpine pretreatment in animals.

Procedure. Two men and two women between the ages of 19 and 27 were selected for study. All subjects were in good health and were found to be normal on physical and laboratory examination, including a determination of protein bound iodine.

On each of 5 occasions all subjects were hospitalized under controlled conditions on a clinical research ward. On each occasion the blood pressure response of each subject was determined in the following manner. The subject reclined in a comfortable position and a saline infusion was started in the right arm. The intravenous tubing was connected to bottles containing dilute norepinephrine and dilute tyramine which could be administered at will at a desired rate without the subject's knowledge. A blood pressure cuff and stethoscope bell were attached to the patient's left arm. When blood pres-

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sure had stabilized, the patient was given 2 μg 1-norepinephrine in 20 seconds. His systolic blood pressure was determined at 15 second intervals for 4 minutes. After 15 minutes, when baseline values had been re-established, dl-tyramine was given, one milligram per minute for 4 minutes. Systolic blood pressure was determined during this time for an additional 4 minutes at 15 second intervals.

Testing was done intially to establish baseline norepinephrine and tyramine responses for each subject. Following this, each subject took oral alpha methyldopa, 2 g daily for 7 days. Testing was repeated. Twenty-five days later baseline responses to norepinephrine and tyramine were reestablished. Each subject then took one milligram of reserpine orally for 28 days. Norepinephrine and tyramine testing was repeated. Following this, 2 subjects were given oral DOPA and 2 were given placebo in an identical capsule in double blind fashion. DOPA was given 100 mg the first day and 600 mg daily for 4 days. The norepinephrine and tyramine pressor responses of all 4 subjects were then performed for the fifth and final time. The experiment was single blind except for the DOPA procedure.

Analysis of data. The point of interest was change in blood pressure after injection of tyramine or norepinephrine. It was thus desirable to eliminate any influence of the subjects' general or "absolute" levels of blood pressure and to have the scores represent strictly changes after amine injection. Consequently, each subject's baseline blood pressure reading was subtracted from his subsequent pressure reading for each trial. Such scores for each drug (reserpine, methyldopa, and DOPA) were then compared to corresponding scores for responses during the preceding control period. This was done for both infused substances (tyramine and norepinephrine). The statistical steps were analyses of variance of the "mixed model" type, involving 2 fixed-constant variables ("drug *vs* control" and "measurement periods") and one random sampling variable (the subjects). Similar designs are discussed in McNemar(3) and Lindquist(4). Such analyses yield information about the influence of a number of main effects and interactions, but only the effect of "drug by period" interaction is of major interest in this study. When significant, this effect means that the shape of the blood pressure curves during control and during drug conditions are reliably different. The statements of significance in the following description of the results are based on the "drug by period" interaction F ratio.

Results. Since 2 control periods were ob-

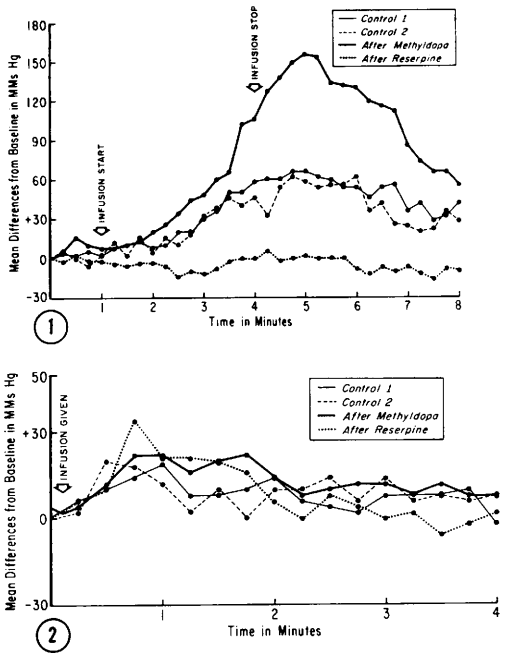

FIG. 1. Systolic Blood Pressure Response to Tyramine

tained with both norepinephrine and tyramine, it was possible to compare controls as a check on both degree of experimental control and possibility of changes due to time, increasing familiarity with the procedures, etc. The analyses for both infused substances yielded very small F ratios, both for shapes of curves and for total elevation. This suggested that the experiment was reasonably controlled and that time effects were not present.

A. Pressor response to tyramine. Changes in response to tyramine infusion are shown in Fig. 1. The curves represent the mean

change above baseline in millimeters of mercury during (and just following) infusion of tyramine. Statistical analysis revealed that while the control curves were highly similar (as previously stated), the curves under the methyldopa and reserpine conditions were reliably different from their respective control curves. The probabilities that the differences between curve shapes were due to random variation or "error" were less than one in one thousand for both drugs. F ratios were 4.41 for methyldopa and 2.52 for reserpine with 31/93 degrees of freedom with $p < .001$ in each case.

Five days after cessation of reserpine only one subject showed recovery of pressor response to tyramine, and this subject, during the 5 day interval, had been taking placebo, not DOPA. On inspection the "DOPA *vs* placebo" results did not show sufficient differential effect to justify statistical analysis.

B. Pressor response to norepinephrine. Fig. 2 shows the mean change above baseline in millimeters of mercury after infusion of norepinephrine. The control curves were again very similar. The methyldopa curve was not considered reliably different from its control (F ratio < 1.00). Reserpine pretreatment *did* produce a blood pressure response different from its control ($p < .01$, F ratio 3.00, with 15/45 degrees of freedom). The results after "DOPA *vs* placebo" were not sufficiently different on inspection to require statistical analysis.

Discussion. A. Methyldopa influence on tyramine response. Methyldopa is one of the newer antihypertensive agents(5,6) and its mechanism of action has been under considerable scrutiny. Several authors attribute its action to reduced norepinephrine biosynthesis secondary to competitive inhibition of the decarboxylase enzyme system(7,8,9). It competes not only with DOPA but also with tryptophan, tyrosine, phenylalanine, and histidine (8). Both brain and heart tissue levels of norepinephrine show depletion after methyldopa treatment(10-12).

Tyramine exerts a pressor effect through the release of endogenous, stored norepinephrine(2,13). This would lead one to anticipate a diminished pressor response to infused tyramine after pretreatment with methyldopa, as the latter drug depletes tissue stores. However, *enhanced* pressor response has been found in hypertensive patients. Neither was the *expected* diminution seen with our normal subjects. Rather, pressor enhancement was clearly demonstrated, just as in hypertensive patients.

Although there is no entirely satisfactory explanation for this phenomenon, there are 3 main possibilities. 1) Methyldopa may sensitize adrenergic receptors. This seems unlikely since present data have shown infused norepinephrine to be no more potent after methyldopa pretreatment. 2. The metabolic product of methyldopa, alpha methyl norepinephrine, which is a weak pressor, may be of such nature, or stored in such manner, that relatively great quantities are liberated by infused tyramine. No available data bear on this possibility. 3) The weak pressor alpha methyl norepinephrine may have relatively profound pressor action (after tyramine-induced release) due to decreased degradation. It is known that methyldopa is metabolized to alpha methyl norepinephrine and this has one-third the vasopressor activity of norepinephrine in man(1,14). The ability to restore a diminished tyramine response in reserpinized humans by methyldopa and to restore the response in reserpinized animals with methyl dopamine and methyl norepinephrine to and beyond pre-reserpine levels was demonstrated by Pettinger *et al* (1). These data indicate that methyl norepinephrine is not a substrate for monoamine oxidase (MAO) activity.

Competition may occur between methyl norepinephrine and norepinephrine for storage sites. Even so, the question would remain: why does a less potent vasopressor produce increased pressor response? As stated, methyl norepinephrine is not a substrate for monoamine oxidase (MAO) breakdown(1). The alternate metabolic pathway, catechol o-methyl transferase (COMT) would appear to be the chief pathway for methyl norepinephrine degradation. Axelrod (15) states that COMT has been found widely in all mammals. The major enzymatic activity site is the liver and COMT is the

primary pathway for breakdown of circulating norepinephrine thereby being important in terminating its pressor action. However, there is significant physiologic MAO inactivation of norepinephrine within the storage site prior to any pressor activity(16). Methyl norepinephrine, not an MAO substrate, would avoid this source of destruction and would have to be o-methylated. This would seem to occur, in large part, after exerting stimulatory effect on adrenergic receptors. The similarity of our two controls would seem to indicate that a given dose of tyramine will empty a constant number of storage pools. If as we hypothesize, the pools are partially filled with alpha methyl norepinephrine (with one-third vasopressor potency), then the enhancement seen would indicate that a major portion of tyramine-released norepinephrine is MAO inactivated prior to contact with adrenergic receptors.

B. Reserpine influence on tyramine response. Reserpine is known to deplete tissue levels of norepinephrine in humans and animals(17,18,19). The degree of depletion appears dose related(19). Kopin and Gordon (16) have demonstrated that reserpine decreases binding capacity and exposes norepinephrine to MAO activity. Harrison *et al* (17), measuring serum and tissue levels of norepinephrine in dog hearts, found a decrease in tissue levels without measurable changes in the serum levels of norepinephrine after reserpine suggesting that the degradation of norepinephrine, after reserpine, occurs completely within the storage pool via MAO activity. We observed the expected ablation of pressor response to infused tyramine after 28 days of reserpine treatment as shown in Fig. 1. This confirmed the effectiveness of oral therapeutic doses of reserpine (1 mg daily) in depletion of norepinephrine stores.

C. Methyldopa influence on norepinephrine response. Fig. 2 points out our finding of no significant alteration in response to infused norepinephrine after methyldopa and is in agreement with previous studies(20,21). This indicates no apparent change in peripheral receptor sensitivity and no alterations in COMT activity potential with a very small dose (2 μg) of norepinephrine.

D. Reserpine influence on norepinephrine response. Fig. 2 shows statistically significant enhanced pressor response to infused norepinephrine after therapeutic range, oral doses of reserpine. This phenomenon was observed in animals by Burn and Rand(2) using much larger doses of reserpine and norepinephrine. They felt receptor sensitization was the cause and after infusing norepinephrine over a period of time, were able to restore previous response levels. Prange *et al* (22) demonstrated pressor enhancement in humans to an equally small dose of norepinephrine as ours (2 μg) after imipramine pretreatment. They hypothesized the implication of storage impairment. Present data with long term reserpine administration do not obviate the possibilities of storage site disassemblement or of a decrease in potential COMT activity.

E. "DOPA *vs* placebo" effects after reserpine. DOPA has been shown to reverse the tranquilizing behavioral effects of reserpine in animals(23,24). Friend(25) showed a marked rise in plasma levels of norepinephrine after a single dose of DOPA which returned to normal in 4 hours. Klerman *et al* (26), working with depressed patients, did not find sustained serum elevations of catecholamines with chronic DOPA administration and did not find demonstrable changes in the depressive symptomatology. Burn and Rand(2) showed in animals a return of pressor response to tyramine, after reserpinization, by infusing norepinephrine over a short period of time. They found, too, that tissue levels were repleted. We anticipated recovery of the pressor response to tyramine to pre-reserpine levels by repletion facilitated with DOPA.

The 2 subjects receiving DOPA and one placebo subject showed no evidence of repletion. One placebo subject regained his pre-reserpine response. It would seem prudent to pursue further studies of this phase using a larger subject population, large doses of DOPA, and establishing control data for each subject as to his speed of physiological repletion without drugs and then with DOPA.

Summary. The pressor effects of small doses of norepinephrine and tyramine have

been studied in normal humans after pretreatment with therapeutic range doses of methyldopa, reserpine, and DOPA. 1) Methyldopa pretreatment causes an enhanced response to tyramine. 2) Reserpine pretreatment causes a diminished response to tyramine. 3) Methyldopa pretreatment does not affect response to norepinephrine. 4) Reserpine pretreatment causes an enhanced response to norepinephrine. 5) After reserpine, DOPA treatment has no reliable effect on either norepinephrine or tyramine response.

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