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# PHARMACOLOGIC STUDIES WITH 7-BENZYL-1-ETHYL-1,4-DIHYDRO-4-OXO-1,8-NAPHTHYRIDINE-3-CARBOXYLIC ACID[1]

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ABSTRACT

ACETO, M. D., L. S. HARRIS, G. Y. LESHER, J. PEARL AND THEODORE G. BROWN, JR.: Pharmacologic studies with 7-benzyl-1-ethyl-1,4-dihydro-4-oxo-1,8-naphthyridine-3-carboxylic acid. J. Pharmacol. Exp. Therap. **158**: 286–293, 1967. 7-Benzyl-1-ethyl-1,4-dihydro-4-oxo-1,8-naphthyridine-3-carboxylic acid (NCA) is a potent locomotor stimulant in a variety of laboratory animals. The drug was studied to determine the pertinent pharmacology and possible mechanism of action. NCA was compared with *d*-amphetamine and pipradrol in locomotor activity experiments in combination with reserpine and α-methyl-*dl*-tyrosine. Additional motor activity studies involving combinations of NCA with phenoxybenzamine and *p*-chlorophenylalanine were also done. Reserpine blocked NCA-induced and pipradrol-induced hypermotility whereas it only reduced *d*-amphetamine-induced hypermotility. α-Methyl-*dl*-tyrosine antagonized the activity of *d*-amphetamine but only partially blocked pipradrol and was completely ineffective *vs.* NCA. Phenoxybenzamine antagonized and *p*-chlorophenylalanine had no effect on the NCA-induced hypermotility. The mechanism of action of NCA appears to be dependent upon catecholamines and the drug may act by altering norepinephrine uptake or by releasing norepinephrine from the storage pool. These studies also suggested different mechanisms of action for *d*-amphetamine and pipradrol.

Initial pharmacologic studies in mice and rats with 7-benzyl-1-ethyl-1,4-dihydro-4-oxo-1,8-naphthyridine-3-carboxylic acid (NCA; see structural formula shown in fig. 1) indicated that this drug was a potent locomotor stimulant and that unusually high doses were required to produce death. Additional studies established the fact that NCA also caused intense locomotor stimulation in a wide variety of species such as gerbils, cats, dogs and monkeys (Aceto *et al.*, 1966). The intent of the present studies was to determine additional pharmacologic properties and the possible mechanism of action of this naphthyridine derivative. Comparisons with positive controls such as *d*-amphetamine and pipradrol greatly assisted this purpose.

METHODS. *Drugs.* The drugs and sources were as follows: *d*-amphetamine sulfate, Mann Research Laboratories, Inc., New York, N.Y.; NCA, synthesized by Dr. G. Lesher and Miss P. Brundage in this laboratory; *p*-chloro-*dl*-phenylalanine, synthesized by Dr. N. Albertson in this laboratory; α-methyl-*dl*-tyrosine, (α-MT), Cyclo Chemical Corporation, Los Angeles, Calif.; α-methyl-*l*-tyrosine, courtesy of Dr. B. Witkop, Bethesda, Md.; pipradrol hydrochloride, courtesy of Wm. S. Merrell Company, Cincinnati, Ohio; reserpine injection, Philadelphia Ampoule Laboratories, Philadelphia, Pa.; pentobarbital sodium, Pentobrocanal, Winthrop Laboratories, Rensselaer, N.Y.; and phenoxybenzamine hy-

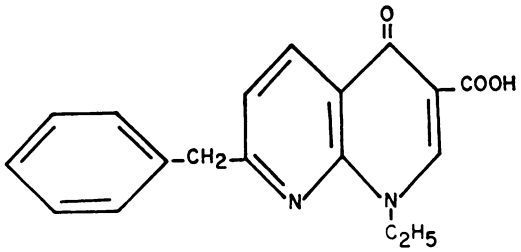


FIG. 1. Structural formula of NCA.

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drochloride, courtesy of Smith, Kline & French Laboratories, Philadelphia, Pa.

*Spontaneous motor activity in mice.* The method used was a modification of that reported by Harris and Uhle (1961) and originally described in principle by Dews (1953). The units were 40.5 cm in diameter and 10 cm in height and were housed in a sound-attenuated and temperature-controlled (22–24°C) room. Digital counters outside the test room recorded the number of photocell interruptions for 30 min. Experimentally naive male ICR mice (Windy Acres Farms) in the weight range of 20 to 24 g were allowed free access to food (Wayne Lab Blox) and water. Then, 30 min before being randomly assigned to one of 12 photocell activity units, each group of four mice per photocell unit was medicated subcutaneously, except where otherwise noted, with drug (dose expressed as drug base). Locomotor activity was measured during the 30- to 60-min period after the administration of *d*-amphetamine, pipradrol or NCA to compare the drugs during the time in which the most stable and equivalent locomotor activity was evident. For example, after 60 min, the intensity of the locomotor activity induced by *d*-amphetamine rapidly subsides. Both pipradrol and NCA are relatively long-acting in this respect compared to *d*-amphetamine. The volume injected was 0.1 ml/10 g. Overt behavioral changes were also noted. Appropriate controls were tested. When NCA was studied in combination with other drugs, the pretreatment time and route of administration for each drug prior to receiving NCA was 3½ hr for *dl*- and *l*-α-MT (i.p.), 2½ hr for phenoxybenzamine (p.o.) and reserpine (i.p.) and 72 hr for *p*-chlorophenylalanine (i.p.). The studies involving combinations of *d*-amphetamine or pipradrol with α-MT or reserpine were similar to the NCA combination studies with respect to pretreatment time and route of administration. All results were expressed as mean 30-min counts ± S.E.

RESULTS. The dose-response curves for *d*-amphetamine and NCA are illustrated in figure 2 and the dose-response curve for pipradrol is shown in figure 3. *d*-Amphetamine and NCA increased locomotor activity with peak effects at 2.5 mg/kg, whereas pipradrol showed a peak effect at 10 mg/kg. The animals were still stimu-

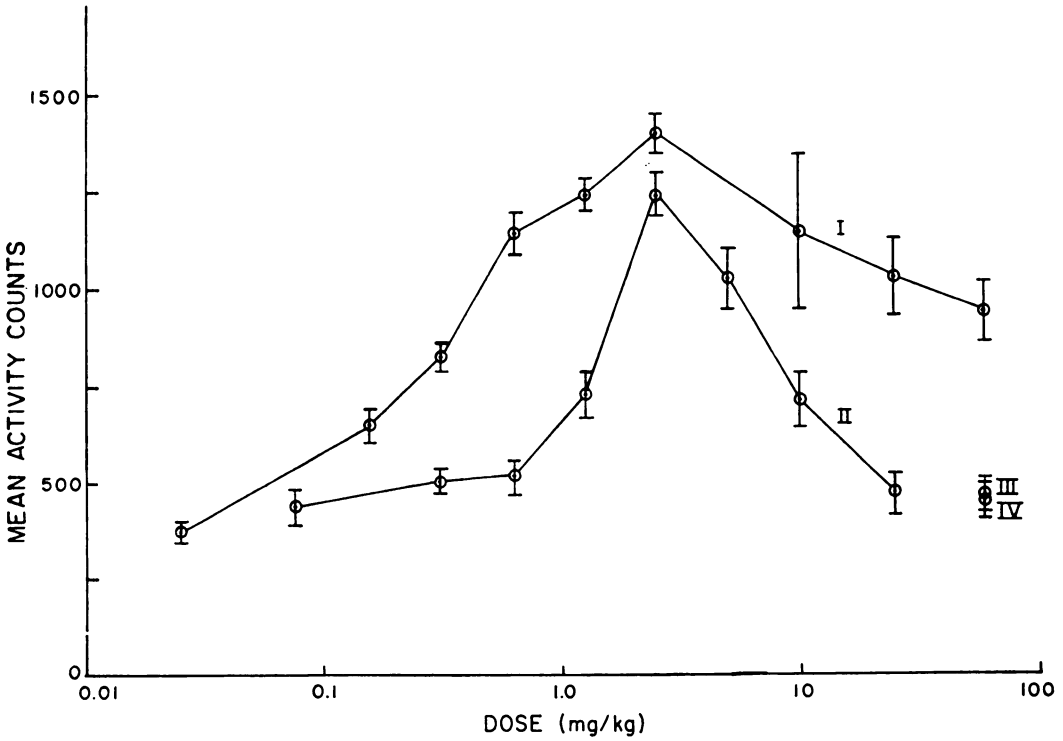


FIG. 2. Effect of NCA and *d*-amphetamine on the spontaneous locomotor activity of mice. Each point represents the 30-min mean count for eight cages (four mice/cage), with the vertical lines indicating S.E. I, NCA; II, *d*-amphetamine; III, $H_2O$ control; IV, GT control.

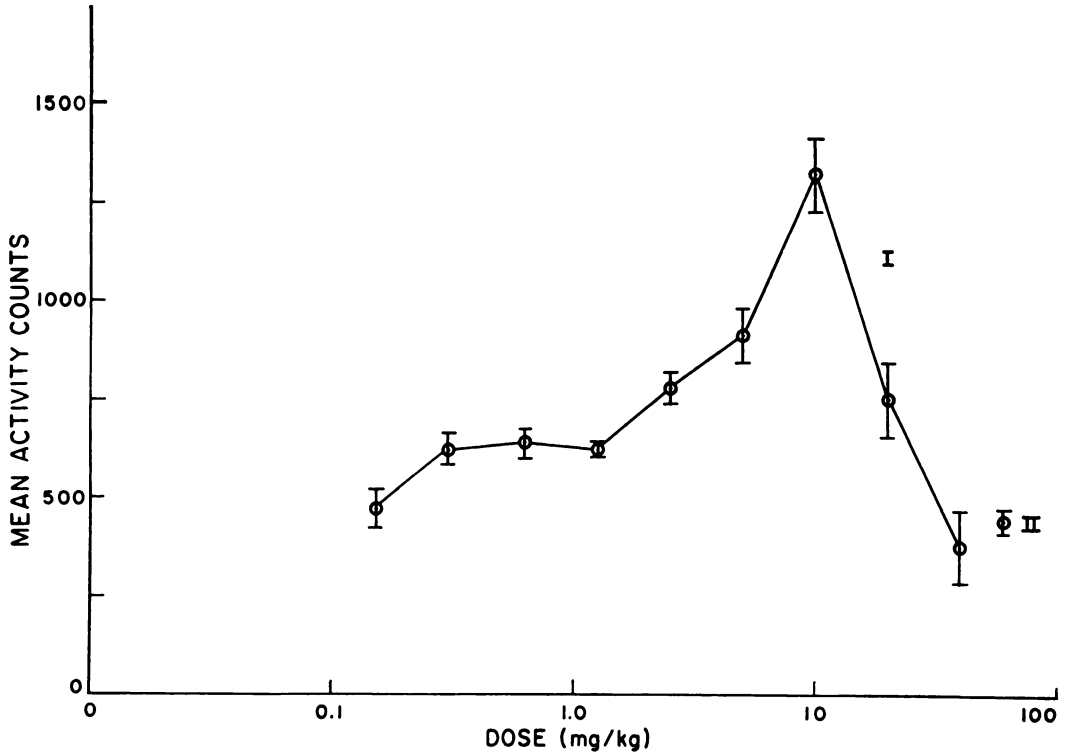


FIG. 3. Effect of pipradrol on the spontaneous locomotor activity of mice. Each point represents the 30-min mean count for eight cages (four mice/cage), with the vertical lines indicating S.E. I, pipradrol; II, $H_2O$ control.

lated at higher doses; however, for all three drugs the activity shifted from locomotor stimulation to other signs of intense stimulation, such as gnawing at the floor grids, vocalizing, biting and other activity not accounted for by the photocell digital counter. No deaths were observed during the testing period for *d*-amphetamine, NCA or pipradrol. NCA was more active than either *d*-amphetamine or pipradrol on a milligram-to-milligram basis over a much wider dose range.

Peak-effect doses were used in all drug combination studies except the one involving *p*-chlorophenylalanine. The results of the studies involving combinations of increasing doses of reserpine with NCA are shown in figure 4. It is apparent that reserpine produces a dose-related blockade of NCA-induced stimulation. It is also apparent that reserpine produces a dose-related blockade of pipradrol, as shown in figure 5. These results contrast with those of parallel studies involving interactions of reserpine and *d*-amphetamine in which the mice were stimulated at all of the dose combinations studied. Van Rossum *et al.* (1962) and Smith (1963) reported enhanced activity in reserpine-treated rodents challenged with *d*-amphetamine, but enhancement was not observed in our studies. It is possible that these differences may be due to the different experimental parameters used. It was reported earlier (Aceto *et al.*, 1966) that NCA reversed akinesia produced by reserpine; however, the highest dose of reserpine used in those studies was 2 mg/kg. When the dose was raised to 10 mg/kg (see fig. 4), it was obvious that reserpine blocked NCA-induced hyperkinesia.

Contrasting results were produced by combinations of *dl*-α-MT plus *d*-amphetamine compared to combinations of *dl*-α-MT plus NCA. As illustrated in figure 6, graded doses of *dl*-α-MT in combination with *d*-amphetamine caused a dose-related reduction of counts. With the exception of one combination (*dl*-α-MT, 16 mg/kg,

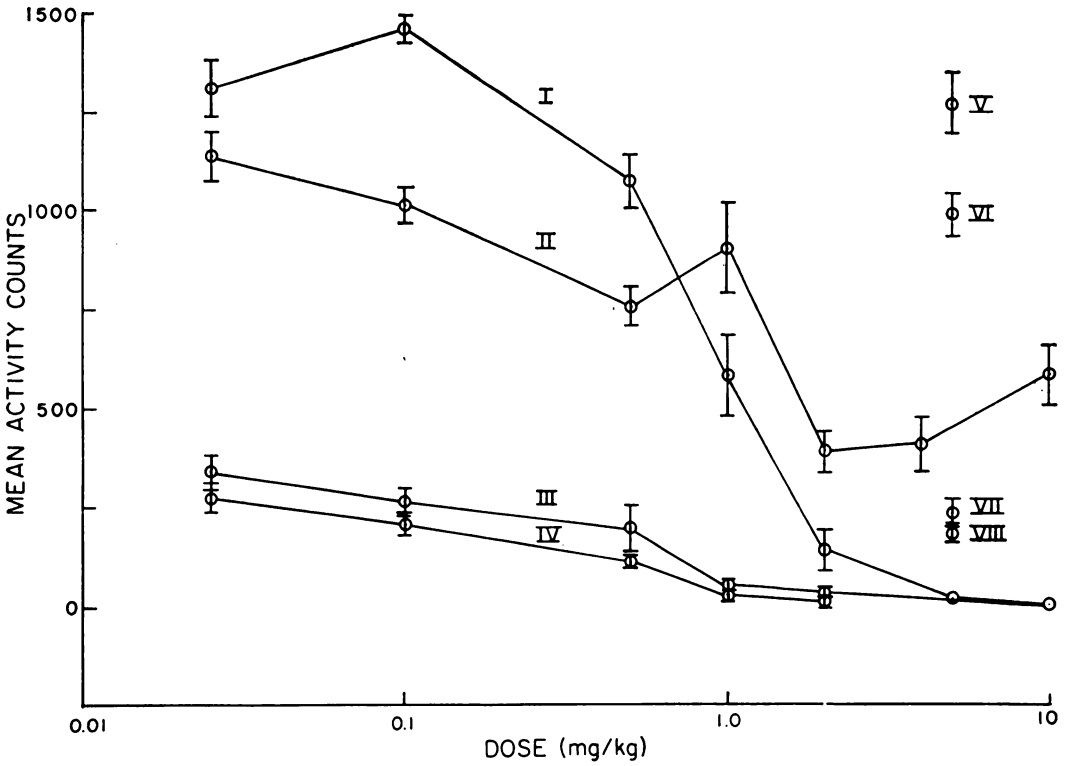


FIG. 4. Effect of reserpine in combination with NCA and in combination with *d*-amphetamine on the spontaneous locomotor activity of mice. Dose of reserpine is indicated on the abscissa. Dose of NCA or *d*-amphetamine was kept constant (2.5 mg/kg, peak-effect dose). I, reserpine plus NCA; II, reserpine plus *d*-amphetamine; III, reserpine plus GT control; IV, reserpine plus $H_2O$ control; V, $H_2O$ plus NCA control; VI, $H_2O$ plus *d*-amphetamine control; VII, $H_2O$ plus GT control; VIII, $H_2O$ plus $H_2O$ control. Each point represents the 30-min mean activity count for eight cages (four mice/cage), with the vertical lines indicating S.E. At the highest doses for reserpine controls and reserpine plus NCA-medicated animals, S.E.'s are not indicated because they are too small. The means are practically zero.

plus NCA), all the other combinations of *dl*-α-MT plus NCA gave results within the range of control values (1% gum tragacanth (GT) plus NCA or GT plus NCA). The results obtained with the *dl*-α-MT plus *d*-amphetamine combinations in mice are essentially in agreement with those reported for *l*-α-MT by Weissman *et al.* (1966); *i.e.*, *dl*-α-MT antagonized the effects of *d*-amphetamine in a dose-related manner over a wide dose range. The *dl*-α-MT plus GT controls showed dose-related impairment of locomotor activity (fig. 6) and the animals also appeared to be sedated, but no toxic effects were obvious. Similar but more potent antiamphetamine effects were noted with *l*-α-MT and all of the dose combinations of *l*-α-MT plus NCA were well within the range of control values. Although overall similar results were noted with either *dl*- or *l*-α-MT, Swiss-Webster mice were used with *l*-α-MT whereas ICR mice were used with *dl*-α-MT. Since ICR mice were used in all the other locomotor activity studies reported here, only the results obtained with *dl*-α-MT are presented. α-MT reduced but did not completely antagonize the motor stimulation produced by pipradrol (fig. 7).

The results of the phenoxybenzamine plus NCA interactions and the results of appropriate controls are illustrated in figure 8. By inspection, it is apparent that phenoxybenzamine antagonizes NCA-induced stimulation in a dose-related manner. Sedation, ptosis and other obvious signs of depression accompanied the reduction of activity.

The combination of *p*-chlorophenylalanine plus NCA did not produce significant differences

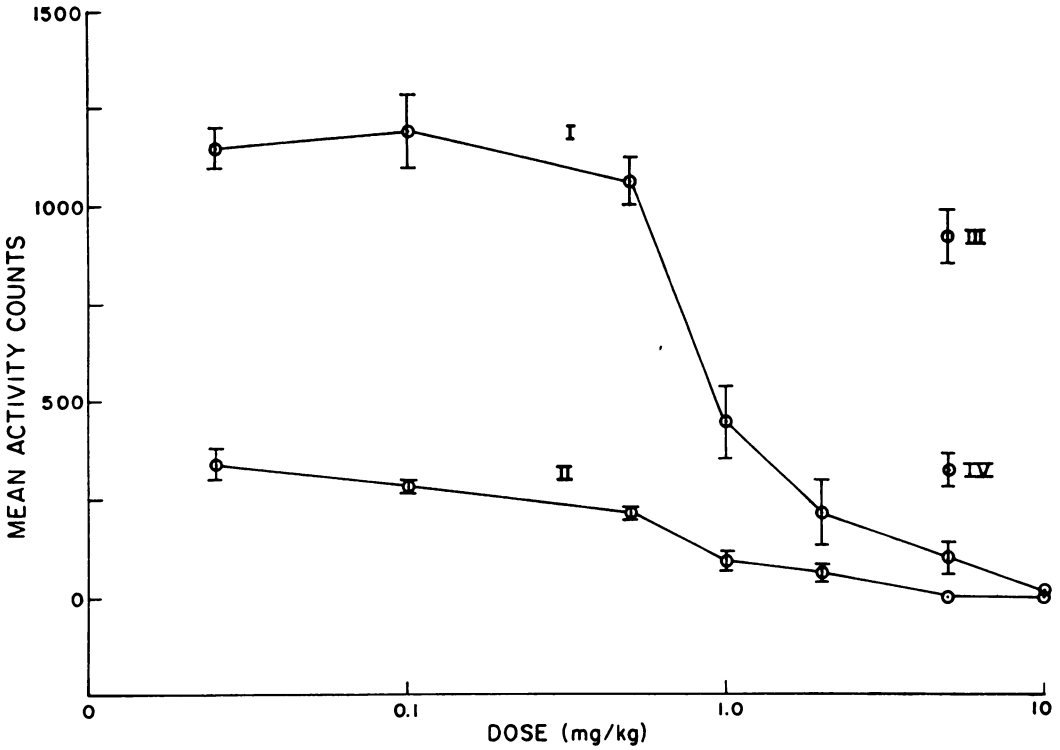


FIG. 5. Effect of reserpine in combination with pipradrol on the spontaneous locomotor activity of mice. Dose of reserpine is indicated on the abscissa. Dose of pipradrol was kept constant at 10 mg/kg (peak-effect dose). I, reserpine plus pipradrol; II, reserpine plus $H_2O$; III, $H_2O$ plus pipradrol; IV, $H_2O$ plus $H_2O$ control. Each point represents the 30-min mean activity count for eight cages (four mice/cage), with the vertical lines indicating S.E. At the highest doses for reserpine controls and reserpine plus pipradrol-medicated animals, the S.E.'s are not indicated because they are too small. The means are practically zero.

when compared to controls (table 1). The dose of *p*-chlorophenylalanine used was the same as that reported by Koe and Weissman (1966) for rats. No overt behavioral changes were seen.

DISCUSSION. Overt observations in a variety of animal species depict NCA as an amphetamine-like drug. Behavioral experiments also show that the drug increased avoidance responses of the rat as has been reported for *d*-amphetamine (Hearst and Whalen, 1963) and pipradrol (Stone, 1960). In spite of the similarities, other toxicity studies on isolated and grouped mice and previously reported LD50's in mice and rats (Aceto *et al.*, 1966) indicate that NCA is much less toxic than *d*-amphetamine.[3]

[3] Supplementary material is deposited as Document no. 9619 with the American Documentation Institute in Auxiliary Publications Project, Photoduplication Service, Library of Congress, Washington, D.C. 20540.

Reserpine has been shown to reduce the levels of a variety of amines such as serotonin, norepinephrine, dopamine and histamine in the central nervous system (Pletscher *et al.*, 1955; Holzbauer and Vogt, 1956; Paasonen and Vogt, 1956; Green, 1964). Although an enormous amount of work has been done to determine the significance of amine levels (Costa *et al.*, 1962; Carlsson *et al.*, 1963; Toman, 1963), most of the studies involved with locomotor activity implicate norepinephrine and/or dopamine rather than serotonin (Smith and Dews, 1962; Van Rossum, 1963; Smith, 1963; Day and Rand, 1963; Matsuoka *et al.*, 1964; Everett and Wiegand, 1962).

Reserpine is known to inhibit the transport of catecholamines (Carlsson *et al.*, 1963), and reserpine is also reported to block the uptake of labeled norepinephrine from the isolated cat brain (Dengler *et al.*, 1962). Since reserpine

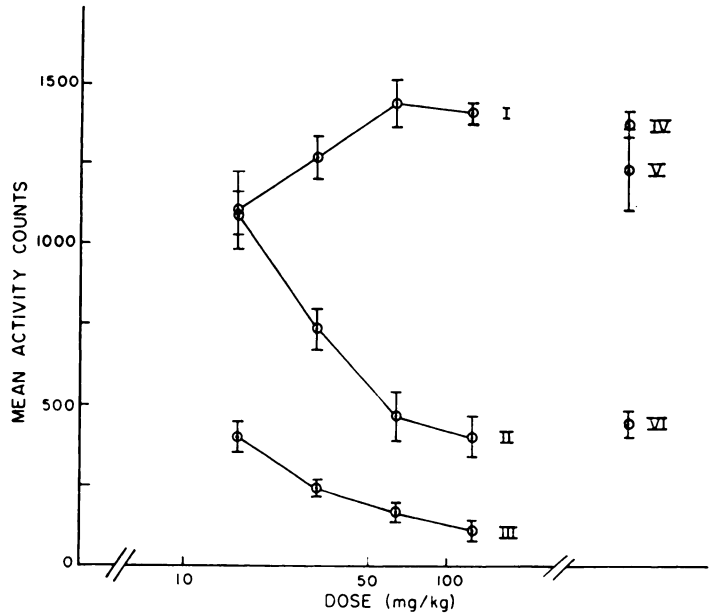


FIG. 6. Effect of α-MT in combination with NCA and in combination with *d*-amphetamine on the spontaneous locomotor activity in mice. Dose of α-MT is indicated on the abscissa. Dose of NCA and *d*-amphetamine was kept constant at 2.5 mg/kg (peak-effect dose). I, α-MT plus NCA; II, α-MT plus *d*-amphetamine; III, α-MT plus GT; IV, GT plus NCA control; V, GT plus *d*-amphetamine control; VI, GT plus GT control. Each point represents the 30-min mean activity count for eight cages (four mice/cage), with the vertical lines indicating S.E.

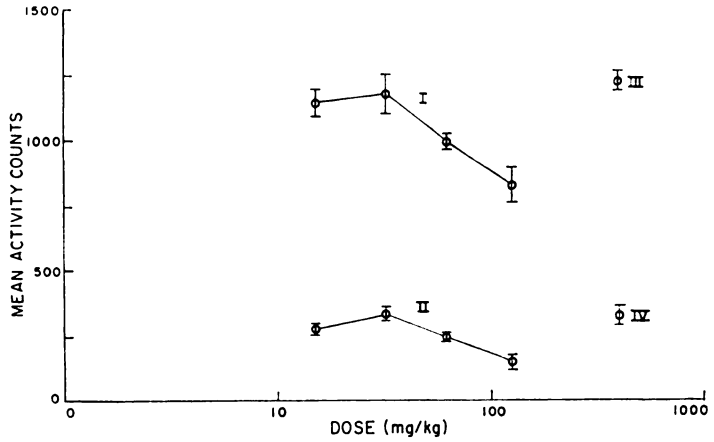


FIG. 7. Effect of α-MT in combination with pipradrol on the spontaneous locomotor activity of mice. Dose of α-MT is indicated on the abscissa. Dose of pipradrol was kept constant at 10 mg/kg (peak-effect dose). I, α-MT plus pipradrol; II, α-MT plus $H_2O$; III, $H_2O$ plus pipradrol; IV, $H_2O$ plus $H_2O$ control. Each point represents the 30-min mean activity count for eight cages (four mice/cage), with the vertical lines indicating S.E.

can completely block NCA-induced stimulation, it appears that the mechanism of action of this naphthyridine derivative is dependent upon catecholamines. The results obtained with phenoxybenzamine, the *alpha*-adrenergic blocker and inhibitor of catecholamine transport (Carlsson *et al.*, 1963), and *p*-chlorophenylalanine, the selective serotonin depletor (Koe and Weissman,

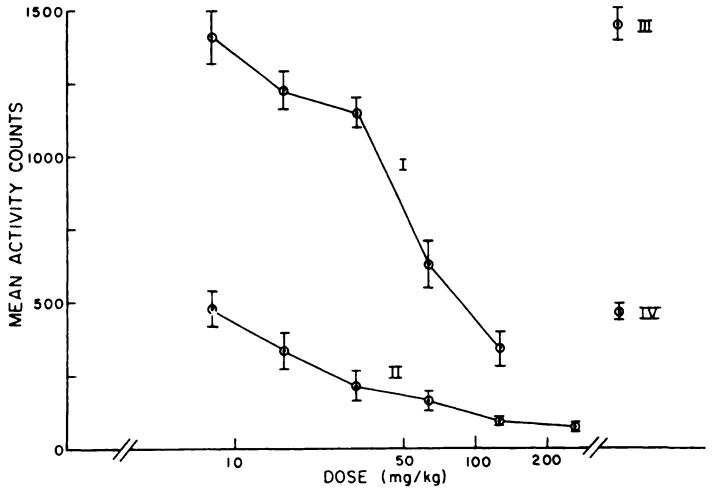


FIG. 8. Effect of phenoxybenzamine in combination with NCA on spontaneous locomotor activity of mice. Dose of phenoxybenzamine is indicated on the abscissa and dose of NCA was kept constant at 2.5 mg/kg (peak-effect dose). I, phenoxybenzamine plus NCA; II, phenoxybenzamine plus GT control; III, GT plus NCA control; IV, GT plus GT control. Each point represents the 30-min mean activity count for eight cages (four mice/cage), with the vertical lines indicating S.E.

TABLE 1

*Effect of p-chlorophenylalanine (p-CP)[a] on NCA-induced hyperkinesia*

| Drug | Activity Count[b] |
|---|---|
| *p*-CP plus NCA[c] | 1207 ± 180 |
| *p*-CP plus GT | 548 ± 68 |
| GT plus GT | 529 ± 40 |
| GT plus NCA | 1266 ± 55 |

[a] Pretreatment of 72 hr and 316 mg/kg i.p.
[b] Results given as 30-min mean ± S.E.
[c] Dose of NCA, 1.25 mg/kg s.c.

1966), support the idea that the amines involved may be catecholamines (probably norepinephrine) rather than serotonin.

The existence of various storage pools for norepinephrine in the central nervous system has been suggested many times (Smith, 1963; Rech, 1964; Glowinski and Axelrod, 1965; Weissman *et al.*, 1966). It has been postulated that the storage pool serves as a reservoir for the functional pool (Crout *et al.*, 1962) and that uptake of norepinephrine is another method of maintaining norepinephrine levels in the functional pool (Blakely *et al.*, 1964).

Nagatsu *et al.* (1964) reported α-MT to be a potent inhibitor of tyrosine hydroxylase, which is believed to be the rate-limiting step in the biosynthesis of catecholamines from tyramine (Hess *et al.*, 1961; Levitt *et al.*, 1965). Spector *et al.* (1965) showed that this compound lowered brain concentrations of norepinephrine and dopamine and felt that the depletion of these amines resulted from the inhibition of tyrosine hydroxylase. Weissman and Koe (1965) and Weissman *et al.* (1966) studied the antiamphetamine effects of α-MT, and Weissman *et al.* (1966) presented evidence "that a critical level of norepinephrine at the receptor is required for amphetamine to exert its customary effects." These authors also speculated "that this level derives from a functional pool of norepinephrine in the central nervous system, highly susceptible to blockade of norepinephrine biosynthesis at the tyrosine hydroxylase step." The studies involving combinations of pipradrol plus reserpine and pipradrol plus α-MT suggest that the mechanism of action of pipradrol is also dependent upon catecholamines; however, the degree of the involvement of the various pools of norepinephrine remains to be determined. Our studies involving interactions of α-MT plus NCA were revealing because α-MT did not block the hypermotility and other stimulatory effects produced by NCA. It is speculated that the mechanism of action of NCA is dependent upon catecholamines. The evidence suggests that the drug may alter the rate of uptake of norepinephrine or release norepinephrine from the reserve pool.

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