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NEUROPSYCHOPHARMACOLOGICAL INVESTIGATIONS WITH
4H-3-METHYLCARBOXAMIDE-1,3-BENZOXAZINE-2-ONE:
DEMONSTRATION OF A NOVEL SPECTRUM
OF CENTRAL ACTIVITY

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NEUROPSYCHOPHARMACOLOGICAL INVESTIGATIONS WITH 4H-3-METHYLCARBOXAMIDE-3,4-BENZOXAZINE-2-ONE: DEMONSTRATION OF A NOVEL SPECTRUM OF CENTRAL ACTIVITY

By

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ABSTRACT

NEUROPSYCHOPHARMACOLOGICAL INVESTIGATIONS
WITH 4H-3-METHYLCARBOXAMIDE-3,4-BENZOXAZINE-2-ONE:
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Since the fortuitous discovery of the mood elavating effects of the monoamine oxidase inhibitor (MAOI) iproniazid and the tricyclic antidepressant (TCA) imipramine in the mid-1950's, there has been a persistent search for new and improved antidepressant agents lacking the bothersome side effects and toxicities assiciated with the MAOI and the TCA. A new and apparently efficacious antidepressant agent, caroxazone, was compared with various, MAOI and TCA for effectiveness and potency to protect against reserpine-induced and α-methyltyrosine-induced depression of a learned motor skill, to potentiate the psychomotor stimulant and toxic effects of concomitant administration of (L)3,4-dihydroxyphenylalanine and a peripheral decarboxylase inhibitor, to inhibit brain type-A MAO activity in vitro and for effects on the concentration of norepinephrine in the hypothalamus using rats as subjects. The results strongly suggest that the central pharmacodynamic profile of caroxazone is fundamentally different from that of either the TCA of the MAOI.

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PREFACE

"As Bokonon invites us to sing along with him:

If you wish to study a 'grandfalloon',
Just remove the skin of a toy balloon."

Kurt Vonnegut, Jr.

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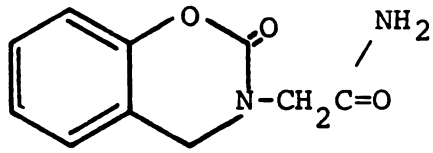
INTRODUCTION

General Introduction for Thesis

INTRODUCTION

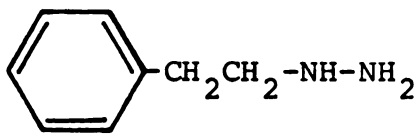
Modern psychopharmacology came into being in 1950 with the syntheses of the phenothiazine neuroleptic chlorpromazine (Delay and Deniker, 1952). Psychotherapeutic agents for the treatment of affective disorders also became available in the 1950's when the mood elevating effects of iproniazid were noticed in patients suffering from tuberculosis. The inhibition of monoamine oxidase (MAO; monoamine; O_2 oxidoreductase; EC 1.4.3.4) by iproniazid was first described by Zeller et al. (1952). Iproniazid was not introduced into general use in the treatment of depressive disorders until 1957 and many other monoamine oxidase inhibitors (MAOI) that are efficacious in the treatment of depressive syndromes have been described since then. Figure 1 shows the chemical structure of some once widely used MAOI antidepressants.

A second class of antidepressants, the tricyclic antidepressants (TCA), also came into being in the 1950's as a result of molecular modifications of some of the earlier antihistaminic drugs. The antidepressant efficacy of this class of drugs was quickly recognized and their use rapidly became widespread. Figure 2 shows the chemical structures of some TCA and of the phenothiazine nucleus.

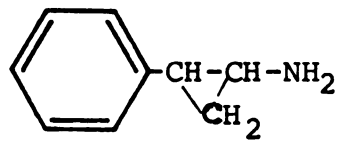


CAROXAZONE

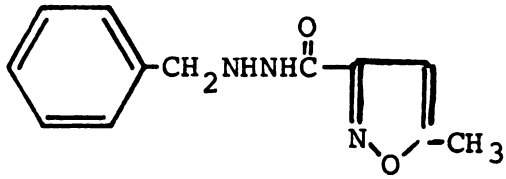
4H-3-methylcarboxamide-1,3-benzoxazine-2-one



PHENELZINE

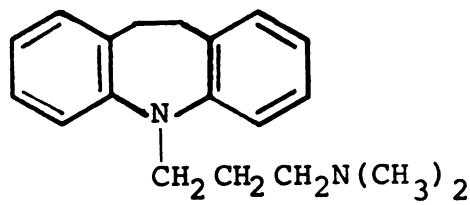


TRANYLCYPROMINE

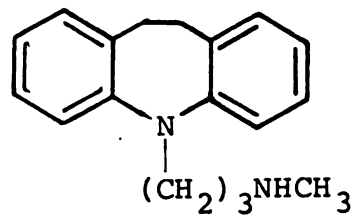


ISOCARBOXAZID

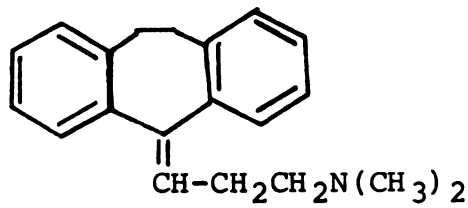
Figure 1. Chemical structure of caroxazone and some monoamine oxidase inhibitors (MAOI).



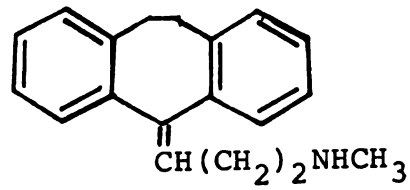
Imipramine



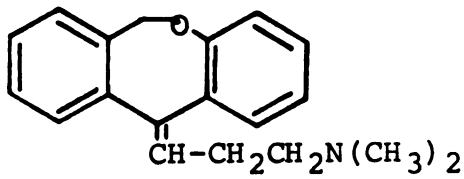
Desipramine



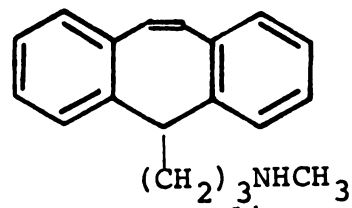
Amitriptyline



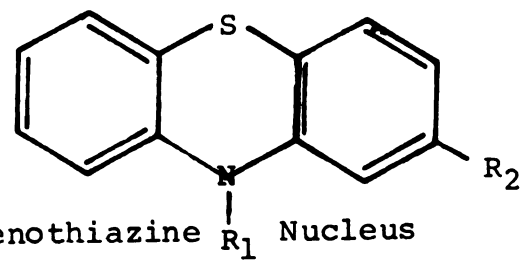
Nortriptyline



Doxepin



Protriptyline



Phenothiazine Nucleus

Figure 2. Chemical structures of some tricyclic antidepressants and of the phenothiazine nucleus.

The MAOI drugs are a rather heterogeneous group of compounds in that they exert many different effects and have various chemical structures. All of the MAOI that are clinically efficacious in the treatment of endogenous depression exert a prolonged suppression of monoamine oxidase activity after *in vivo* and *in vitro* administration (Neff and Goridis, 1972; Moore, 1971; Planz *et al.*, 1972; Tipton, 1972; Eiduson, 1972; Youdim and Sandler, 1967; Youdim, 1972a, 1972b; Spector *et al.*, 1963). In this respect these compounds constitute a relatively homogeneous drug class in that only minor differences in the duration of drug effects exist. Monoamine oxidase is believed to exist in multiple forms (i.e., isoenzymes) in the mammalian central nervous system (CNS) and other tissues as well (Collins *et al.*, 1970; Sandler and Youdim, 1972; Youdim, 1967, 1972a, 1974; Youdim *et al.*, 1974; Youdim and Sandler, 1967). The significance of multiple forms of cerebral MAO in clinical depression and its treatment with MAOI, even the existence of these forms, has been debated in much of the current literature (Jain, 1977; Fuller, 1972; Neff and Yang, 1974; Fuentes and Neff, 1975; Maitre *et al.*, 1976; Planz, 1972; Sandler and Youdim, 1974; Housley *et al.*, 1976; Neff and Goridis, 1972; Waldmeier *et al.*, 1976; Waldmeier and Maitre, 1975). Differences in substrate affinities for different MAO types seen *in vitro* may be of little significance to *in vivo* antidepressant activities. For example, there are negligible qualitative differences between the MAOI in animal antidepressant screening tests

at doses that maximally suppress brain level of enzyme activity and bring about changes in the concentrations of brain monoamine neurotransmitters and their metabolites (Everett, 1967; Hill and Tedeshi, 1971; Iversen and Iversen, 1975; Moore, 1971; Rech, 1975; Maitre et al., 1976).

MAOI pretreatment will prevent a number of behavioral effects due to reserpine (Blaschko and Chruschiel, 1960; Smith, 1962), potentiate the stimulant effects of indirect-acting central nervous system (CNS) stimulants such as amphetamine (Scheckel et al., 1969), potentiate the CNS stimulant effects of L-DOPA and L-tryptophan (Everett, 1967; Rech and Thut, 1976; Creveling et al., 1968; Everett, 1957, 1967, 1970; Spector, 1967; Thut, 1970; Grahame-Smith, 1974; Wiegland and Perry, 1961) and prevent the general behavioral depression associated with tyrosine hydroxylase inhibition by α-methyltyrosine (Moore and Rech, 1967). MAOI reverse the depressed affect in a certain proportion of patients suffering from endogenous depression. But MAOI also lack specificity in perturbating brain monoamine systems, many side effects and interactions resulting from enzyme inhibition in peripheral nerves and the liver. The complications associated with chronic suppression of MAO activity in hepatic and cardiovascular tissues are widely appreciated. MAOI-induced hepatotoxicity, impaired red-green color vision and neurologic damage are believed to be unrelated to MAO inhibition (Neff and Yang, 1976). Because of these serious side effects many clinicians view the routine use of this class of antidepressants to be a

questionable practice.

The clinician's choice of antidepressant therapy is presently limited to drugs that are classified as having either the MAOI or tricyclic antidepressant (TCA) types of activity, as defined by various laboratory procedures (Hill and Tedeschi, 1971; Askew, 1965; Iversen and Iversen, 1975; Rech, 1974, 1975; Sigg, 1959, 1965; Sulser, 1961a, 1962). The effects of MAOI on the concentration of 5-HT, NE and DA metabolites in the CNS is not different from what one would expect (Yang and Neff, 1974). Figures 3, 4 and 5 show synthetic and degradative pathways for 5-HT, NE and DA. Enzyme abbreviations are given in the figure legend. Dramatic increases in the 3-0-methylmetabolites of the two catecholamines occur following MAOI treatment. The decreased brain levels of the carboxylic and alcoholic metabolites of DA, 5-HT and NE are also very pronounced. However, the altered levels of these metabolites does not correlate well with the duration of MAOI bahavioral "antidepressant" effects in laboratory animals. On the other hand, the time course of altered 3-0-methyl-catecholamine metabolite levels correlates well with the duration of "antidepressant" activity (Waldmeier et al., 1976; Maitre et al., 1976).

TCA are clinically efficacious and exhibit fewer deleterious side effects than MAOI, and currently are the drugs of choice (Honigfeld, 1973; Goodman and Gilman, 1975; Bielski and Friedel, 1978; Payson, 1971). Drugs classified as TCA are reported to inhibit the synaptic reuptake process

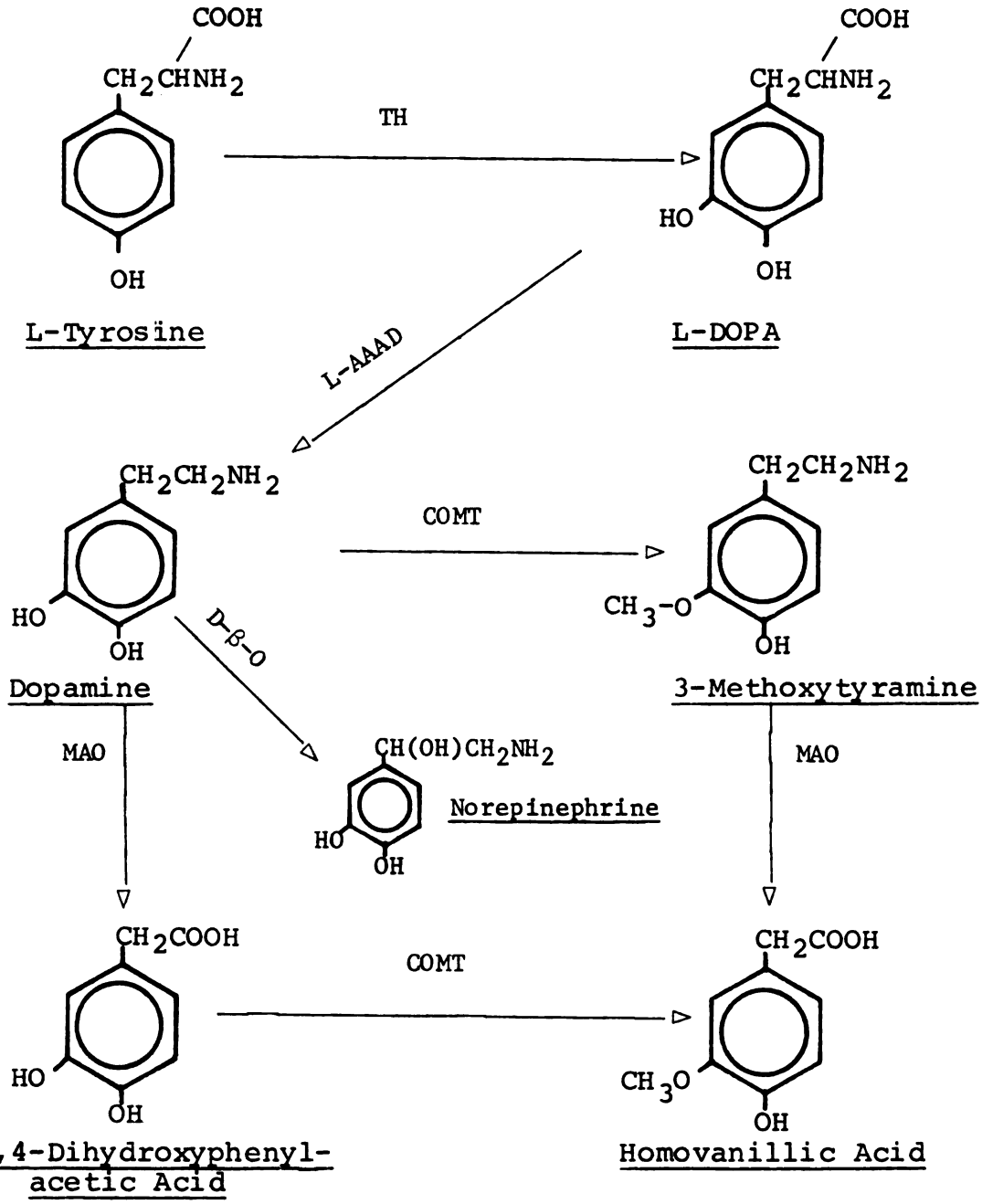


Figure 3. Biochemical pathways for synthesis and degradation of dopamine. Abbreviations: L-AAAD, L-aromatic amino acid decarboxylase; COMT, catechol-O-methyltransferase; D-β-O, dopamine-β-oxidase; MAO, monoamine oxidase; TH, tyrosine hydroxylase.

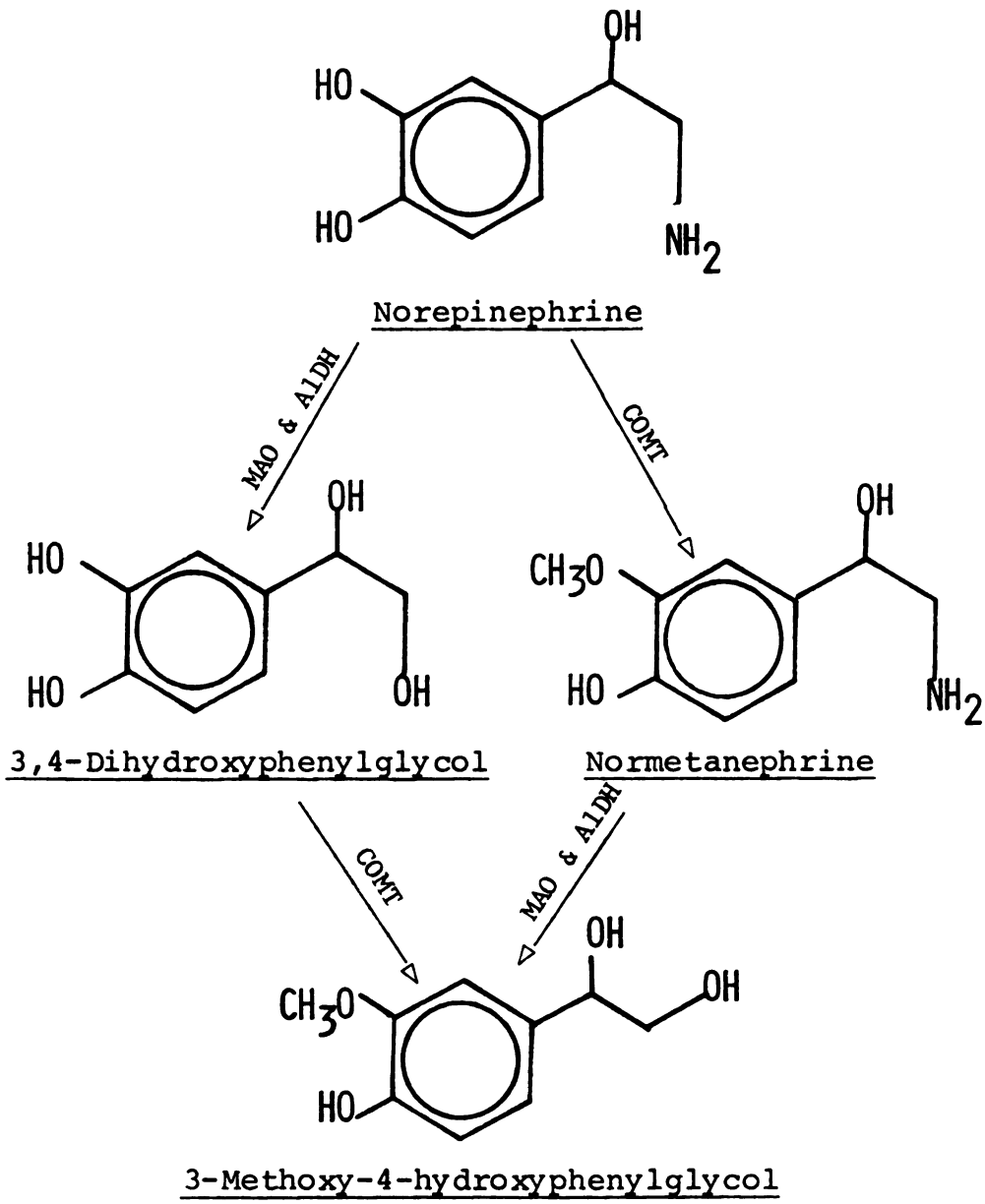


Figure 4. Major metabolic pathways for norepinephrine. Abbreviations: COMT, catechol-O-methyltransferase; MAO, monoamine oxidase; AlDH, alcohol dehydrogenase.

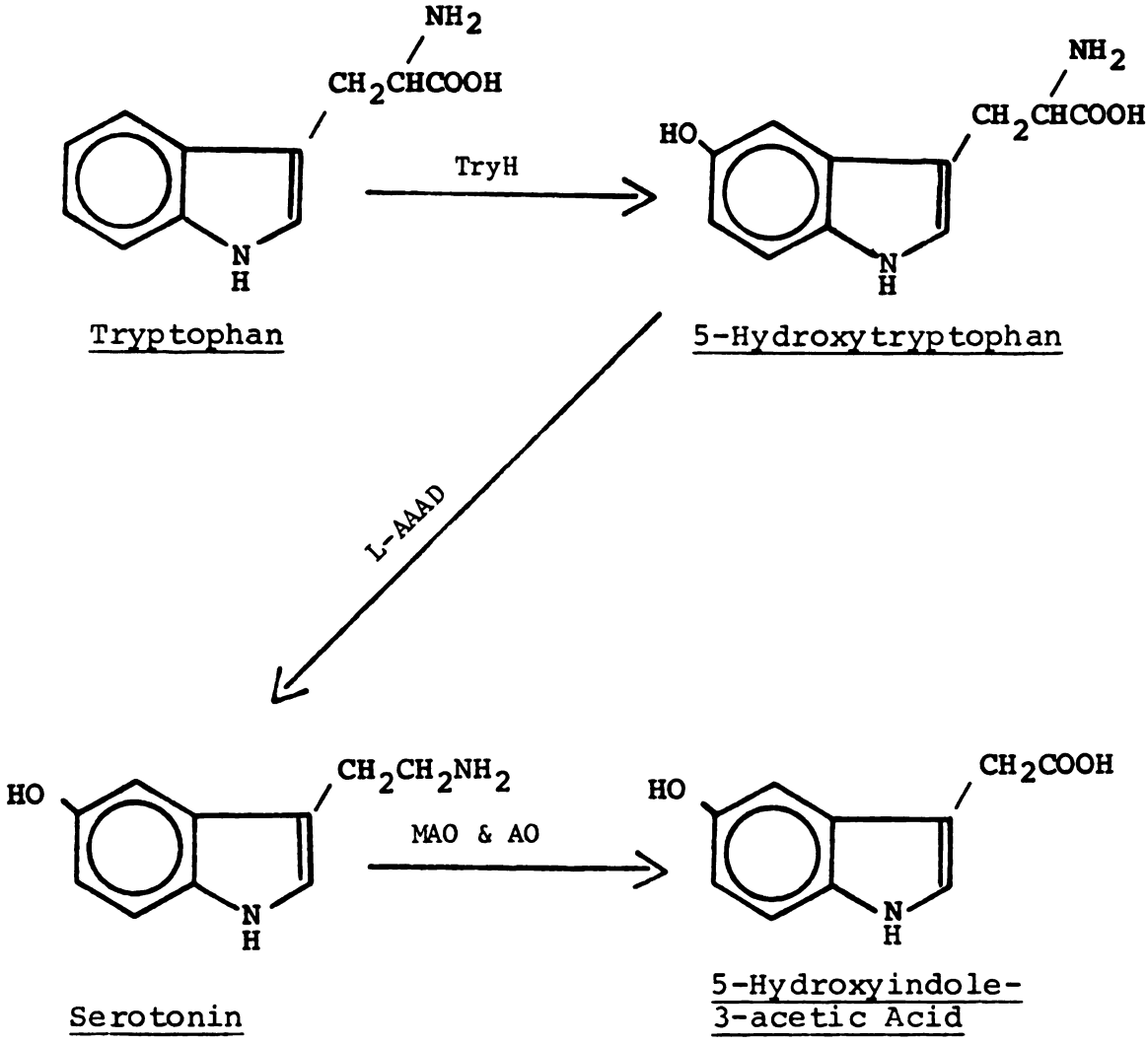


Figure 5. Synthesis and metabolism of serotonin (5-HT). Abbreviations: TryH, tryptophan hydroxylase; MAO, monoamine oxidase; AO, aldehyde oxidase; L-AAAD, L-aromatic amino acid decarboxylase.

at serotonergic and noradrenergic synapses in the mammalian CNS (Carlsson et al., 1968, 1969a, 1969b, 1969c; Corrodi and Fuxe, 1968, 1969; Schildkraut et al., 1969; Schubert et al., 1970; Meek and Werdinius, 1970; Alpers and Himwich, 1972). Such studies suggested that those tricyclic antidepressants which have tertiary amines in their side chains, such as amitriptyline or chlorimipramine, are more potent blockers of, and thus preferentially inhibit, the uptake mechanisms for central serotonergic neurons. In contrast, those antidepressants of the secondary amine type, such as desmethylimipramine, preferentially inhibit uptake mechanisms for central noradrenergic neurons (Carlsson et al., 1969a, 1969b, 1968; Fuxe and Ungerstedt, 1968; Shaskan and Snyder, 1970; Dubinsky et al., 1973). Chronic administration of those antidepressants effecting 5-HT uptake processes retards central 5-HT turnover and decreases brain levels of 5-HT and 5-HIAA (Corrodi and Fuxe, 1968; Schildkraut, 1969c; Schubert et al., 1970; Meek and Werdinius, 1970). It has been postulated that these effects are mediated through some feed-back mechanism which reduces impulse flow in these 5-HT neurons (Corrodi and Fuxe, 1969; Schubert, 1970). The literature on secondary amine tricyclic antidepressant effects on central catecholamine metabolism is less consistent. Although some investigators have reported decreases in the turnover of NE (Glowinski and Axelrod, 1966; Schanberg et al., 1967), others have failed to confirm this observation (Corrodi and Fuxe, 1968; Schubert, 1970; Corrodi et al., 1967). However, secondary amine tricyclic

antidepressants do potentiate the effects of any drug which increases NE levels in the synaptic cleft (Scheel-Krueger, 1972). These observations are interesting since abnormalities in the release of one or more of the catecholamine and indolamine putative CNS neurotransmitters have been seriously implicated as possible disorders behind the clinical manifestations of affective disorders (Davis, 1970; Schildkraut, 1970). The emphasis of basic laboratory and clinical investigations into the psychopharmacology of antidepressants reflects this prejudice.

MAOI offer an alternative treatment in patients not responsive to TCA and are effective in some of these refractory cases. There is considerable interest in finding new drugs with antidepressant efficacy that do not have the potential toxicities of the MAOI, but have a broader therapeutic spectrum and less troublesome side effects than the TCA compounds (anticholinergic, antihistaminic, antiserotonergic).

A new series of drugs, the 1,3-benzoxazines, synthesized by Farmitalia Research Laboratories in Milan, Italy, have been screened for antidepressant properties (R.A. Carrano, personal communication; Suchowsky, 1969c, 1969d). Of the agents studied to date, 4H-3-methylcarboxamide-1,3-benzoxazine-2-one (caroxazone) has shown the most promise. Pharmacological tests in a variety of animal species indicate that caroxazone exhibits many of the drug interactions associated with clinically useful antidepressants (Suchowsky et al., 1969a, 1969b; R.A. Carrano, personal communication).

Initial clinical trials also indicate that this drug shows considerable promise as an antidepressant.

Caroxazone increases brain catecholamine and 5-hydroxytryptamine levels at doses that are in the same range as those showing "antidepressant" activity in the functional screening tests in animals. This is also true for the classical MAOI. The potency of this drug is also in the same range as the more commonly used MAOI (phenelzine, tranylcypromine). Thus, *in vivo* potency and spectrum of activity of caroxazone in these tests would suggest a mechanism similar to that of the MAOI. However, when caroxazone was administered *in vivo*, analysis of MAO activity of brain homogenates did not show a reduction in enzyme activity (Suchowsky *et al.*, 1969b). Repeated treatment with caroxazone was found to *increase* 5-HIAA levels (R.A. Carrano, personal communication). Caroxazone does not appear to have any antihistaminic, anticholinergic or antiserotonergic activity, and therefore would not be expected to cause some of the adverse side effects seen with the TCA (i.e., xerostomia, constipation, blurred vision, etc.). Caroxazone also differes from a TCA in a lack of effect on norepinephrine or 5-hydroxytryptamine uptake (Suchowsky *et al.*, 1969a). Caroxazone does not exhibit amphetamine-like CNS stimulatory activity (Suchowsky *et al.*, 1969a, 1969c). This observation is important since *d*-amphetamine and amphetamine-like CNS stimulants also exhibit many of the drug interactions associated with clinically efficacious antidepressant

agents (i.e., anti-reserpine activity: Rech, 1964, 1975; Pirch, et al., 1967; Moore, 1971; Hill and Tedeschi, 1971; Iversen and Iversen, 1975; McKearney, 1968; Rech and Stolk, 1970). The research effort presented here was designed to better characterize the pharmacodynamics of caroxazone as they relate to "antidepressant" activity. These studies have been organized into 5 sections. The first three sections describe experiments which compared caroxazone with other clinically efficacious antidepressants for activity to elicite "antidepressant" activities as defined by three different experimental procedures. The fourth section describes experiments comparing some of these drugs for potency and effectiveness to inhibit rat brain MAO in vitro, using dopamine as the enzyme substrate. The last section describes the results of experiments comparing phenelzine and caroxazone for activity to influence the concentration of norepinephrine in the hypothalamus of the rat. Each of the sections contains a brief introduction, description of experimental protocol in a methods section, a results section and a discussion section (or a combined results and discussion section).

SECTION I

Antagonism of Reserpine-Induced Behavioral Depression: Comparison of Phenelzine, Isocarboxazid, Desipramine, Amitriptyline, Imipramine, Methylphenidate, d-Amphetamine, Cocaine and Caroxazone

SECTION I

INTRODUCTION

Reserpine treatment produces a wide variety of effects when administered to laboratory animals, most of which are easy to observe and quantitate (i.e., decreased spontaneous motor activity, hypothermia, impairment of various learned behaviors, etc.). One property shared by MAOI and TCA is the ability to both reverse (i.e., the antidepressant drug administered after reserpine) and prevent (the antidepressant administered before reserpine) certain components of the reserpine depressant spectrum. Caroxazone is reported to also exhibit anti-reserpine activity in a variety of species (Suchowsky et al., 1969a, 1969b). For example, caroxazone and imipramine are reported to be approximately equipotent in reversing reserpine-induced hypothermia, decreased spontaneous motor activity and ptosis, and, in general, caroxazone exhibits anti-reserpine activity that is very similar to that seen for wellknown antidepressants. Reserpine depletes central stores of norepinephrine (NE), dopamine (DA) and 5-HT (Alpers and Shore, 1967). Pretreatment with a MAOI will prevent the depletion, whereas TCA are without preventative effects in this particular situation (Tipton, 1972; Christmas et al., 1972; Pirch, 1967; Rech, 1975; Iversen and Iversen, 1975; Moore, 1971).

Pretreatment with caroxazone will also prevent reserpine-induced depletion of cerebral biogenic amine stores (R.A. Carrano, personal communication; Suchowsky, 1969b).

The following study examines the anti-reserpine effects of caroxazone, MAOI, TCA and some CNS stimulant drugs (i.e., methylphenidate, cocaine and d-amphetamine). These drugs were included in the study since d-amphetamine has been used in man, although without much success, as a short-term antidepressant (Payson, 1971). Cocaine effects in this experimental situation were not known and this was another consideration. The results contained in this section have been submitted for journal publication (Vrbanac et al., 1978a).

METHODS

Experimental methods generally follow previously published procedures for examining various drug interactions using rotarod performance in the rat (Rech et al., 1966; Moore and Rech, 1967).

Subjects

All subjects used in this study were female Sprague-Dawley rats weighing from 200 to 250 g. Subjects were purchased from Spartan Farms, Haslett, Michigan, and maintained in laboratory animal facilities with controlled temperature (22°C), humidity (45 %) and diurnal lighting (i.e., 12 hour light cycle from 7 a.m. to 7 p.m.). Purina Rat Chow and water were available ad libitum.

Drugs

The following drugs were used for this study: reserpine alkaloid (S.P. Penick and Co., New York, N.Y.), 4-H-3-methylcarboxamide-1,3-benzoxazine-2-one (caroxazone, free base; Farmitalia Research Laboratories, S.P.A., Milan, Italy), phenelzine (sulfate salt; Warner-Lambert Research Institute, Morris Plains, N.J.), isocarboxazid (Hoffman-LaRoche, Inc., Nutley, N.J.), tranylcypromine (sulfate salt; Smith, Kline and French Laboratories, Philadelphia, Pa.), desipramine (hydrochloride salt; Merrell National Laboratories, Inc.,

Cincinnati, Ohio), imipramine (hydrochloride salt; Geigy Pharmaceutical Division, Ardsly, N.Y.), amitriptyline (hydrochloride salt; Merck, Sharp and Dohme, West Point, Pa.), d-amphetamine (hydrochloride salt; Sigma Chemical Co., St. Louis, Mo.), methylphenidate (hydrochloride salt; Geigy Pharmaceutical Division, Ardsly, N.Y.) and cocaine (hydrochloride salt; Mallinckrodt Chemical Works, St. Louis, Mo.). All drugs were dissolved in 0.9 % saline except for caroxazone and reserpine. Caroxazone was suspended in 0.5 % methylcellulose and reserpine was dissolved in glacial acetic acid and diluted with distilled water to a concentration of 2.0 mg reserpine per ml of dilute acetic acid (2-3 % v/v). Solutions used in the construction of the log-spaced dose-response curve for reserpine-induced loss of rotarod performance were prepared by dilution of 4.0 mg reserpine/ml 3.0 % acetic acid stock solution (i.e., 0.32, 0.56, 1.0, 1.8, 2.0 and 3.2 mg reserpine/ml).

Training Procedure

Subjects were trained to walk on a rotating cylinder, or "rotarod" (RR; 5 inches diameter, 9.5 revolutions per minute). An animal was considered to be trained when it remained on the cylinder for 180 continuous seconds. Animals were placed on the cylinder with the head pointing in the direction of rotation and thus were required to learn to turn around, as well as walk forward, on the cylinder. Drug effects in these rats were examined one or two days later. The same procedures and performance criteria were

used during drug testing as for training. Thus, 100 % performance for any animal required that the subject turn around initially as well as remain on the cylinder for a period of 180 seconds. The same rotarod used to train subjects was used for drug testing.

Dosing and Testing Protocol

Four procedures were carried out in the drug tests: acute antagonism, long-term antagonism, acute prevention, and long-term prevention. For the acute antagonism reserpine (2 mg/kg) was administered 4 hours before testing on the rotarod (RR), and the test drugs were administered 2 hours before the RR determination. The protocol used to evaluate the effects of d-amphetamine, methylphenidate and cocaine was slightly different. Two hours following the reserpine treatment subjects were tested on the rotarod and scores recorded. Subjects were immediately treated with one of the three test drugs and the time of injection was recorded for each subject. Only those subjects showing essentially complete loss of rotarod performance were treated (loss of rotarod performance arbitrarily defined as a score of 10 seconds or less on the rotarod). Subjects were tested 20 minutes after receiving the test drug and a second score was recorded. The time course of effects was determined for doses showing activity to antagonize the effects of reserpine treatment.

For the long-term antagonism RR was measured at 2 and 24 hours after reserpine, the test drug was administered

immediately after the 24-hour measurement, and RR was again determined at 26 hours after reserpine. The protocol used to evaluate the effects of d-amphetamine, methylphenidate and cocaine differed from this procedure in exactly the same manner described previously for the acute prevention procedure. Therefore, the last RR measurement was taken 20 minutes after injection of one of the stimulant test doses.

The acute prevention experiment required the injection of the test drug at 4 hours before placing the subjects on the RR, and 2 hours before reserpine (2 mg/kg). The long-term prevention experiments were of 2 types. The first procedure called for the test drug to be injected 2 hours before reserpine (2 mg/kg), followed by RR determinations at 4, 6, 8, 10 and 24 hours after administering the test drug. However, if performance had completely deteriorated to the non-protection level by 8 or 10 hours, subsequent rotarod measurements were cancelled. The second experiment of the long-term prevention type involved the administration of caroxazone, 32 mg/kg, 2 hours before reserpine (2 mg/kg). RR behavior was tested at 4 and 26 hours after caroxazone, but additional injections of 11 mg/kg of caroxazone were given at 4, 8, 12, 16, 20 and 24 hours. These additional doses had been calculated to be a maintenance dose of caroxazone based on first-order elimination and a blood level half-life of 6 hours (R.A. Carrano, personal communication). A control group received 0.5 %

methylcellulose in place of caroxazone at these same times. The 32 mg/kg dose of caroxazone was chosen since this was maximally effective in preventing RR disruption at 2 hours after reserpine, as determined in pilot studies.

Statistical Analysis

All experimental designs contained the appropriate controls. The highest dose of each test drug was examined for possible effects on RR when given with the reserpine vehicle (dilute acetic acid). A reserpine dose-response pattern on RR was also determined at two and four hours, and the time course of effect following 2 mg/kg reserpine was examined at 2, 6, 8, 10 and 24 hours.

Tests for statistical significance were the Mann-Whitney U test for differences between independent samples (one-tailed, $P<.05$) for the majority of the tests. The non-parametric multiple comparisons by simultaneous test procedures, an *a posteriori* test of samples with equal measures based on U, the Wilcoxin-Mann-Whitney U test for multiple comparisons, was applied to time-course studies ($P<.05$). The Wilcoxin signed-ranks test for differences between related samples (two-tailed, $P<.05$) was applied to the long-term antagonism studies.

RESULTS

Reserpine Dose-Response and Time-Course for Rotarod Impairment

Reserpine was administered in log-spaced doses (LDR) ranging from 0.32 to 4.0 mg/kg and effects on rotarod performance (RR) were established 2 hours thereafter. The results obtained at 2 hours are seen in Figure 6. The second LDR was obtained using a RR that turned slightly faster (10.0 revolutions per min). Doses below 1.0 mg/kg (mean RR score at 1.0 mg/kg = 130 sec, on the slower RR, which was used in all subsequent experiments) did not significantly influence RR behavior. A dose of 1.8 mg/kg decreased RR scores to a mean of 8 sec, less than 5 % of control. Thus, the dose response curve is very steep. A dose of 2 mg/kg was chosen for convenience, since earlier studies had been done with this dose and it represents a dose only slightly larger than that producing maximal impairment. Figure 7 shows the time-course for disruption of RR following a single 2 mg/kg injection of reserpine. The large plot demonstrates the onset of RR impairment as determinations made every 20 minutes for 2 hours. The insert plots a longer time-course, to 24 hours. The behavior was completely disrupted by 100 minutes after reserpine and remained maximally impaired for up to 12 hours. By 24 hours

Figure 6. Log-spaced dose-response curve (LDR) for reserpine-induced loss of rotarod performance. Subjects were tested two hours after receiving an i.p. injection of reserpine. Doses tested were 0.32, 0.56, 1.0, 1.8, 2.0, 3.2 and 4.0 mg/kg (n=4). Vehicle alone had no effect on subject performance (not shown). The circles show results obtained with the rotarod used in all subsequent drug tests (9.5 revolutions/min). The second LDR, indicated by squares, was obtained using a rotarod that turned slightly faster (10.0 revolutions/min).

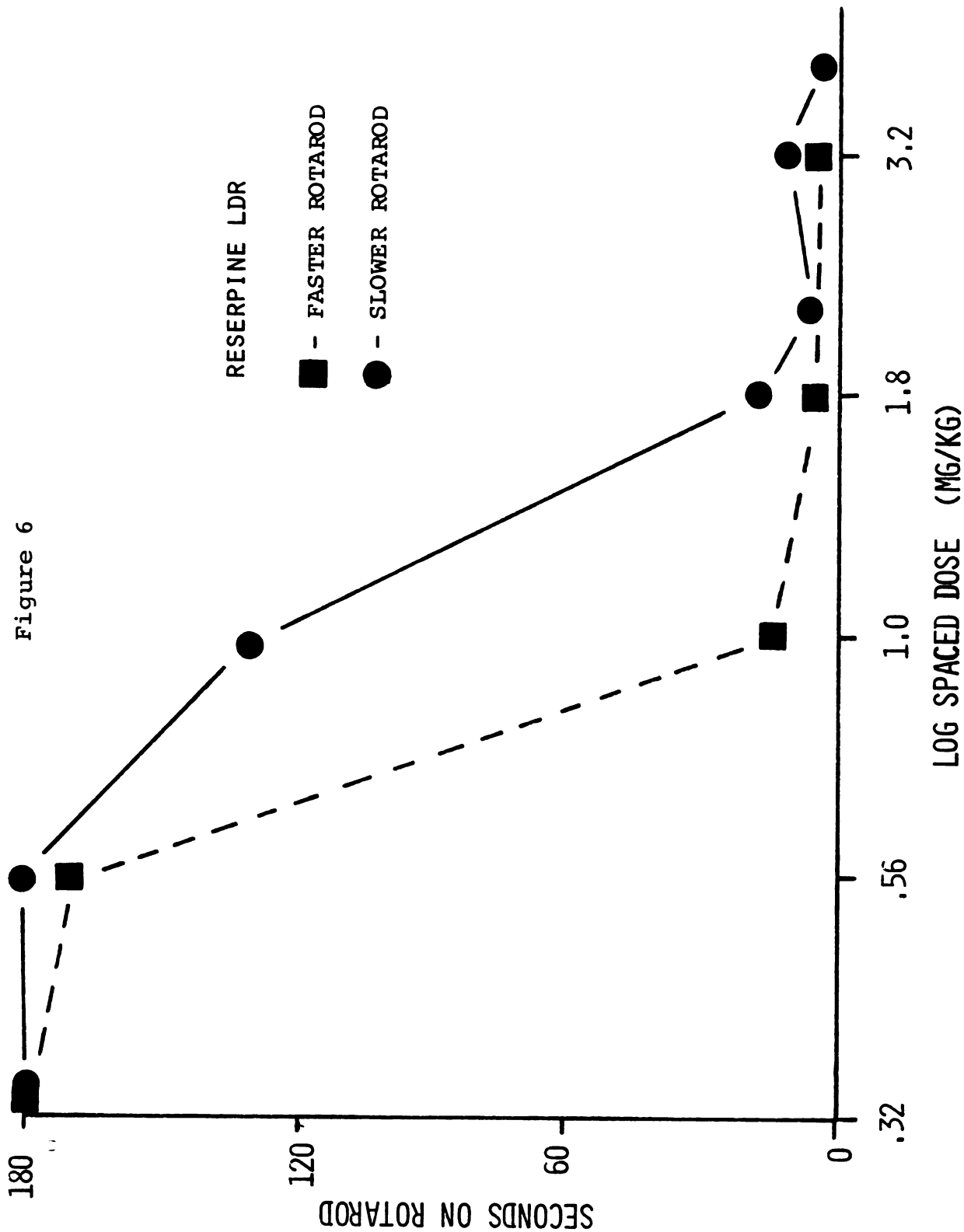
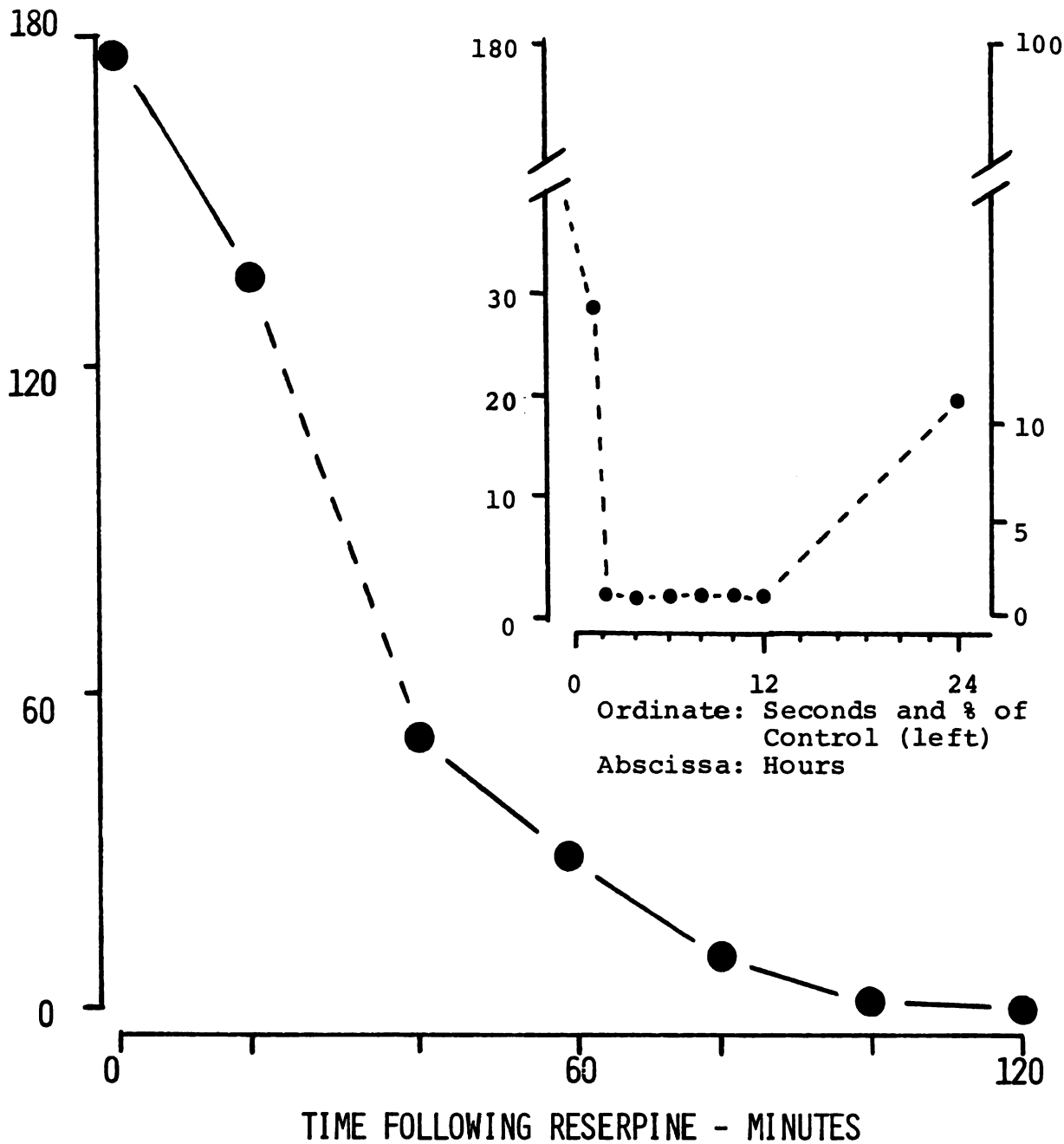

RESERPINE LDR
■ - FASTER ROTAROD
● - SLOWER ROTAROD
SECONDS ON ROTAROD
180
120
60
0
.32
.56
1.0
1.8
3.2
LOG SPACED DOSE (MG/KG)

Figure 6

Figure 7. Time course for reserpine impairment of rotarod performance. Results are expressed as the average time ± S.E.M. in seconds (n=12) on rotarod as determined every 20 minutes for the initial 120 minutes (large plot) and at 4, 6, 8, 10, 12 and 24 hours (displayed in small insert) following 2 mg/kg of reserpine (i.p.). The Mann- Whitney test for multiple comparisons of groups with equal measures was used for statistical analysis. Results for comparison of a) 20, 40, 60, 80, 100 and 120 minute measures and b) 0, 6, 12 and 24 hour measures are displayed in the conventional manner ($p<.05$). Statistical difference between various means is also shown with broken connecting lines, in this and in Figures 3, 4 and 5.

Figure 7


180
120
60
0
0
60
120
TIME FOLLOWING RESERPINE - MINUTES
180
30
20
10
0
100
10
5
0
0
12
24
Ordinate: Seconds and % of Control (left)
Abscissa: Hours

there was only a slight recovery. These results confirm previous observations of reserpine effects on RR (Pirch et al., 1967), and they also parallel in time of onset the depletion of brain monoamines and induction of slow waves in the electrocorticogram.

Acute Antagonism of Reserpine Effects by Antidepressants

The results of the experiments to determine acute antagonism of reserpine effects are listed in Table 1. None of the drugs examined reversed the reserpine decrement in RR. The potential antagonists were also administered after the acetic acid vehicle; at doses listed in Table 1 they did not have a significant effect on RR, the animals performing as well as controls (data not included in Table 1).

Long-term Antagonism of Reserpine Effects by Antidepressants

Groups of 5-6 rats each were trained on the RR to criterion and injected with 2 mg/kg reserpine. The groups were again assessed for RR two hours following reserpine to assure the reserpine impairment (Table 2). At 24 hours after reserpine, the rats were tested for RR and injected immediately thereafter with 10 mg/kg of isocarboxazid, phenelzine, caroxazone, desipramine, or amitriptyline. They were once more tested for RR two hours later (26 hours after reserpine). Comparing the RR scores at 26 hours with those at 24 hours indicated that none of the antidepressant drugs was capable of antagonizing the RR impairment on the second day after reserpine.

TABLE 1

Test for acute antagonism of rotarod decrement 4 hours after reserpine, 2 mg/kg

	Treatment[1]	Seconds on RR, mean ± SD	n
1.	Acetic Acid vehicle	180.0±0.0	12
2.	Reserpine Control[2] (Initial observation)	5.05±2.02	12
3.	Caroxazone 10 mg/kg	4.67±1.97	6
	Paired Control (reserpine alone + drug vehicle)	7.17±7.75	6
4.	Caroxazone 20 mg/kg	4.17±2.93	6
	Paired Control	3.40±0.55	5
5.	Caroxazone 32 mg/kg	6.00±2.00	6
6.	Isocarboxazid 10 mg/kg	11.33±5.15	6
	Paired Control	5.67±1.75	6
7.	Isocarboxazid 20 mg/kg	5.20±2.68	5
	Paired Control	4.67±1.03	6
8.	Tranylcypromine 20 mg/kg	3.20±0.20	5
	Paired Control	5.50±4.23	6
9.	Phenelzine 32 mg/kg	5.10±1.67	6
10.	Desipramine 32 mg/kg	10.50±4.81	6
11.	Amitriptyline 32 mg/kg	5.32±2.59	5

[1]None of the treatments listed significantly antagonized the reserpine impairment of rotarod performance.

[2]Reserpine and paired controls all averaged at 2.8 % of untreated (acetic acid vehicle) performance.

TABLE 2

Test for long-term antagonism of rotarod decrement
24 hours after reserpine

Test Drug[1]	Hours after reserpine, 2 mg/kg			
	0	2	24	26
Isocarboxazid	173.8±6.2[2]	13.7± 5.6	16.2± 4.1	12.7±4.3
Phenelzine	180.0±0.0	49.7±23.0	21.2± 8.2	8.2±2.7
Caroxazone	180.0±0.0	10.0± 5.9	16.8± 5.6	14.0±2.6
Desipramine	180.0±0.0	10.8± 6.2	16.5±11.6	9.0±2.2
Amitriptyline	180.0±0.0	37.2±21.0	6.4± 2.4	11.6±4.2

[1]Each potential antagonist was administered in a dose of 10 mg/kg immediately after the 24-hour RR test. None of the test drugs significantly antagonized reserpine effects at the 26-hour RR test.

[2]Each value is the mean S.E.M. number of seconds that each group (n=5 or 6) walked the rotarod at various times after injection of reserpine.

Acute Prevention of Reserpine Effects by Antidepressants

Listed in Table 3 are the RR scores of groups pretreated with the various antidepressant drugs to assess prevention of reserpine impairment. When caroxazone, phenelzine, isocarboxazid, or tranylcypromine was administered 2 hours before reserpine and RR tested at 4 hours, the usual decrement due to reserpine was completely prevented. Gross observations also clearly indicated that these animals showed no significant signs of having received reserpine. On the other hand, the groups treated with a tricyclic antidepressant (TCA) and the reserpine showed much of the ptosis, hunching, and immobility of the reserpine controls. The larger doses (desipramine and amitriptyline) showed only a very slight protection against the RR disruption by reserpine, and imipramine was ineffective in the dose used.

A more complete dose-response pattern for acute antagonism of reserpine was established for isocarboxazid, phenelzine and caroxazone, as illustrated in Figure 8. Essentially complete protection against reserpine impairment was achieved by isocarboxazid in a dose of 3.2 mg/kg and by phenelzine and caroxazone in a dose of 5.6 mg/kg. Thus, the threshold doses for prevention of reserpine effects among the 3 antidepressants are quite close, although caroxazone is slightly less potent than the other two agents. The caroxazone protection is more variable over the range of higher dosage than in the case of isocarboxazid and phenelzine. Because of this variability a larger number of subjects and doses were employed to determine the caroxazone

TABLE 3

Test for acute prevention of rotarod decrement 2 hours after reserpine

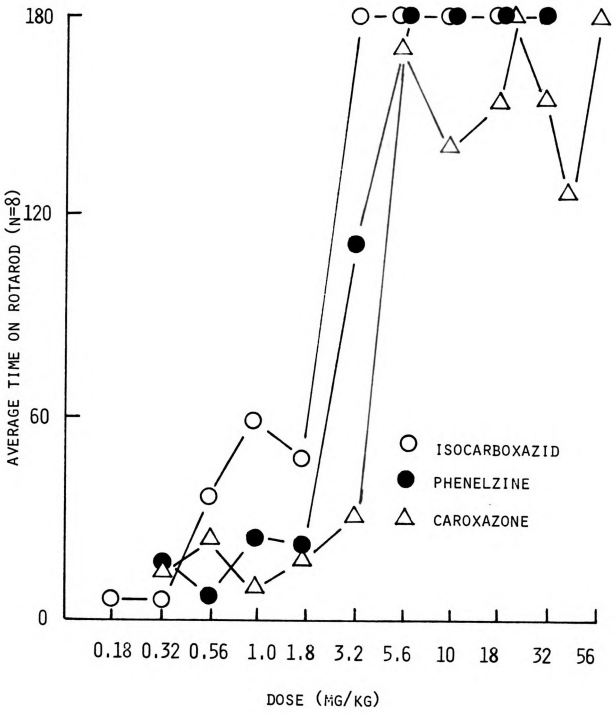
Treatment	Seconds on RR, mean ± S.E.M.	n
1. Vehicle (+ Reserpine 2 mg/kg)	5.20±0.6	20
2. Caroxazone 20 mg/kg	180 ±0.0[1]	5
Caroxazone 40 mg/kg	180 ±0.0	6
3. Phenelzine 18 mg/kg	180 ±0.0	8
Phenelzine 32 mg/kg	180 ±0.0	8
4. Isocarboxazid 20 mg/kg	180 ±0.0	6
Isocarboxazid 40 mg/kg	180 ±0.0	5
5. Tranylcypromine 20 mg/kg	180 ±0.0	6
6. Desipramine 32 mg/kg	11.60±4.0[2]	8
7. Imipramine 18 mg/kg	5.50±1.7	6
8. Amitriptyline 32 mg/kg	18.40±6.2[2]	12

[1] Treatments 2 through 5 were completely effective in preventing reserpine impairment.

[2] These secoes are significantly greater that reserpine controls by the Mann-Whitney U Test, but the effect was just significant at $p < 0.05$.

Figure 8. Four-hour prevention log-spaced dose-response curves for isocarboxazid, phenelzine and caroxazone. Response is the average time in seconds that each group remained on the rotarod when tested 4 hours after antidepressant administration (i.p., and 2 hours after 2 mg/kg reserpine, i.p.). The data displayed for phenelzine and isocarboxazid is the average for 8 subjects except for the 0.56 and 1.8 mg/kg doses of phenelzine (n=7) and the 0.32 and 0.56 mg/kg doses of isocarboxazid (n=6 and 7, respectively). A total of 12 doses of caroxazone was studied with n equal to 7-16 subjects per group.

Figure 8


180
120
60
0
AVERAGE TIME ON ROTAROD (N=8)
ISOCARBOXAZID
PHENELZINE
CAROXAZONE
0.18 0.32 0.56 1.0 1.8 3.2 5.6 10 18 32 56
DOSE (MG/KG)

dose-response curve.

Long-term Prevention of Reserpine Effects by Antidepressant

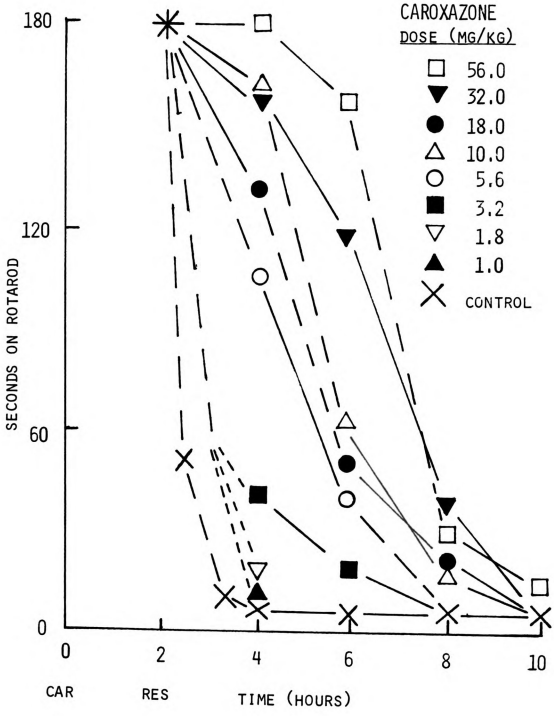
The 3 antidepressant drugs isocarboxazid, phenelzine and caroxazone were compared for long-term protection against reserpine disruption of RR as depicted in Figures 9, 10 and 11. Each antidepressant drug was administered in varying doses two hours before reserpine. RR was measured just before reserpine injection and at 2, 4, 6, 8 and 22 hours after reserpine (4, 6, 8, 10 and 24 hours following antidepressant), or until the protection was completely lost. Figure 9 shows that doses of isocarboxazid of 3.2 mg/kg and greater afford a long-term protection against reserpine impairment of RR, being quite significant although not complete at 24 hours after reserpine. On the contrary, doses of 1.8 mg/kg and less of isocarboxazid exerted a weak and transient protection. Figure 10 indicates a similar pattern for phenelzine in interacting with reserpine. Doses of 5.6 mg/kg and greater protected against the reserpine disruption of RR to a significant degree for at least 24 hours. Phenelzine in doses of 3.2 mg/kg and less afforded partial protection only transiently, up to 4 hours after the depressant. Caroxazone interacted with reserpine in a very different pattern, as seen in Figure 11. The duration of the protective effect was dose-related between 5.6 and 56 mg/kg, but even at the largest dose the effect did not extend beyond 8 hours after reserpine.

Figure 9. Time course of prevention of reserpine depression of rotarod performance following various doses of isocarboxazid. Response is the average time in seconds when tested 4 hours after isocarboxazid and 2 hours after 2 mg/kg of reserpine, with subsequent testing at 6, 8, 10 and 24 hours after isocarboxazid. Each group contained 8 subjects except for the lowest two doses (n=6 for 0.32 mg/kg and n=7 for 0.56 mg/kg). Statistical treatment of the data is described in the Methods section. Statistically significant differences between various means are represented by broken lines ($p<.05$).

Figure 10. Time course of prevention of reserpine depression of rotarod performance following various doses of phenelzine. Response is the average time in seconds when tested 4 hours after phenelzine and 2 hours after 2 mg/kg reserpine, with subsequent testing at 6, 8, 10 and 24 hours after phenelzine. Each group contained 8 subjects except for the 0.56 and 1.8 mg/kg doses (n=7). Statistical treatment is described in the Methods section. Statistically significant differences between various means are represented by broken lines ($p < .05$).

Figure 11. Time course of prevention of reserpine depression of rotarod performance following various doses of caroxazone. Response is the average time in seconds, testing 4 hours after caroxazone and 2 hours after 2 mg/kg reserpine, with subsequent testing at 6, 8 and 10 hours after caroxazone. N=8 for the 1.0, 1.8, 3.2, 5.6 and 18 mg/kg groups. For all other groups n=7. Statistical treatment is described in the Methods section. Statistically significant differences between various means are represented by broken lines ($p<.05$).

Figure 11


CAROXAZONE
DOSE (MG/KG)
56.0
32.0
18.0
10.0
5.6
3.2
1.8
1.0
CONTROL
SECONDS ON ROTAROD
180
120
60
0
0
2
4
6
8
10
TIME (HOURS)
CAR
RES

To determine if caroxazone prevention of reserpine RR disruption could be extended by repeated administration, we carried out the next series of experiments. Caroxazone, 32 mg/kg, was injected 2 hours before reserpine, and RR was measured at 4 and 26 hours after this dose of the antidepressant. In addition, doses of 11 mg/kg of caroxazone were injected at 4-hour intervals after the 32 mg/kg dose, up to 24 hours. A control group received the same treatment, except that caroxazone vehicle was injected in place of the drug. The caroxazone-treated group walked the RR for 174.8±3.1 sec (mean ± S.E.M.) at 4 hours and 108±14.8 sec at 26 hours. The control (reserpine-treated) rats walked the RR for 29.5±11.7 sec at 4 hours and 24.4±9.3 sec at 26 hours. Therefore, maintenance of caroxazone levels over an extended period also extends the protection against the effects of reserpine and yields a pattern of interaction resembling that seen after a single dose of an MAOI.

Acute and Long-term Antagonism of Reserpine Effects by CNS Stimulants

The results obtained for the CNS stimulants are displayed in Figures 12, 13 and 14 and will only be discussed briefly. Figures 12 and 13 show both d-amphetamine and methylphenidate to be effective antagonists of reserpine-induced depression of RR performance, as others have reported and in keeping with the generally accepted profile of amphetamine-like activity (Smith, 1962; McKearney, 1968; Moore, 1971; Rech and Stolk, 1970; Stolk and Rech, 1968;

Figure 12. Time course for acute antagonism of reserpine depression of rotarod performance following d-amphetamine (1 mg/kg), methylphenidate (18 mg/kg) and cocaine (18 mg/kg). Results are expressed as the mean time each group remained on the rotarod (n=8). Significant differences are shown with an asterisk (multiple comparisons by nonparametric simultaneous test procedure of data within each drug group, $p<.05$).

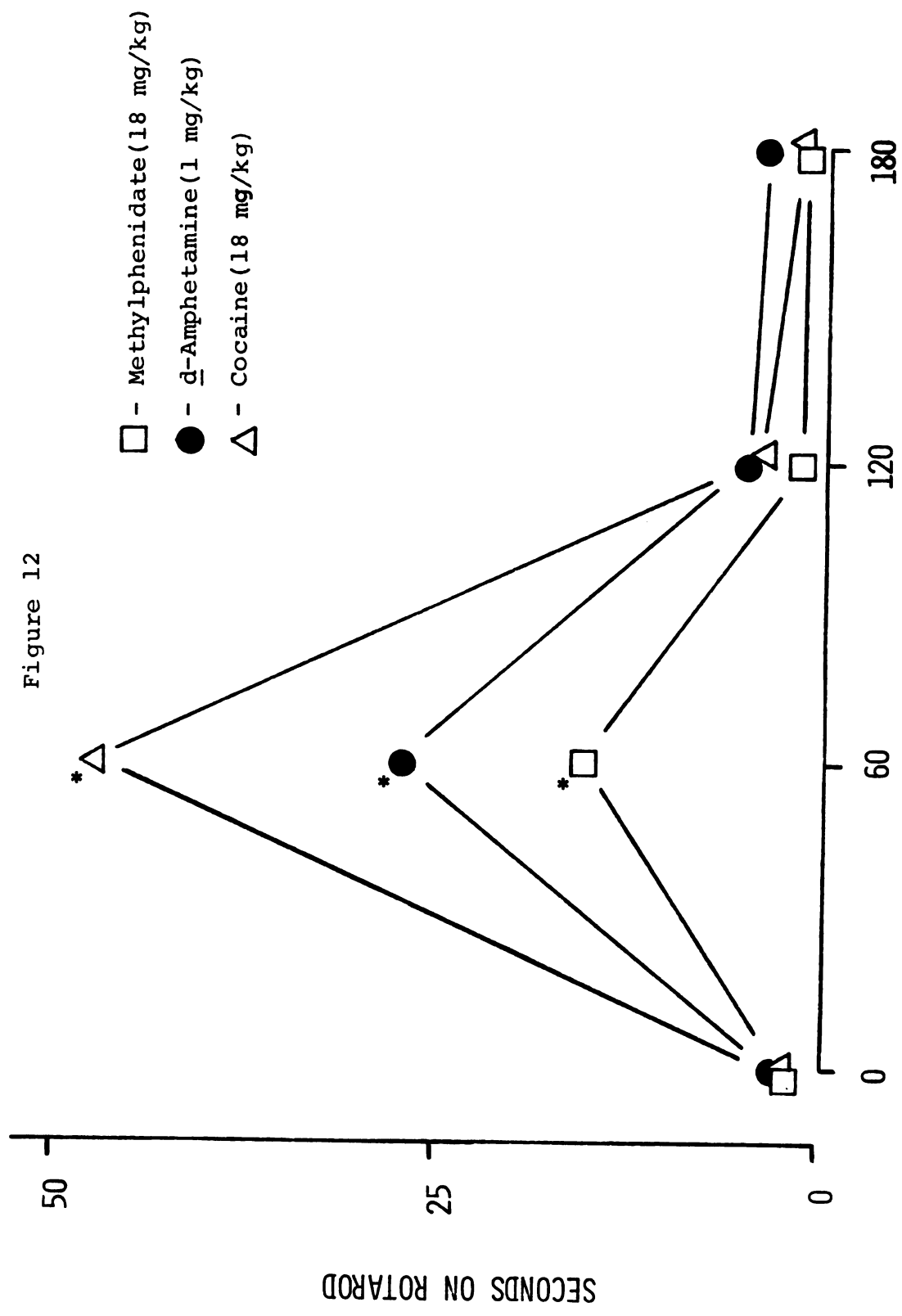
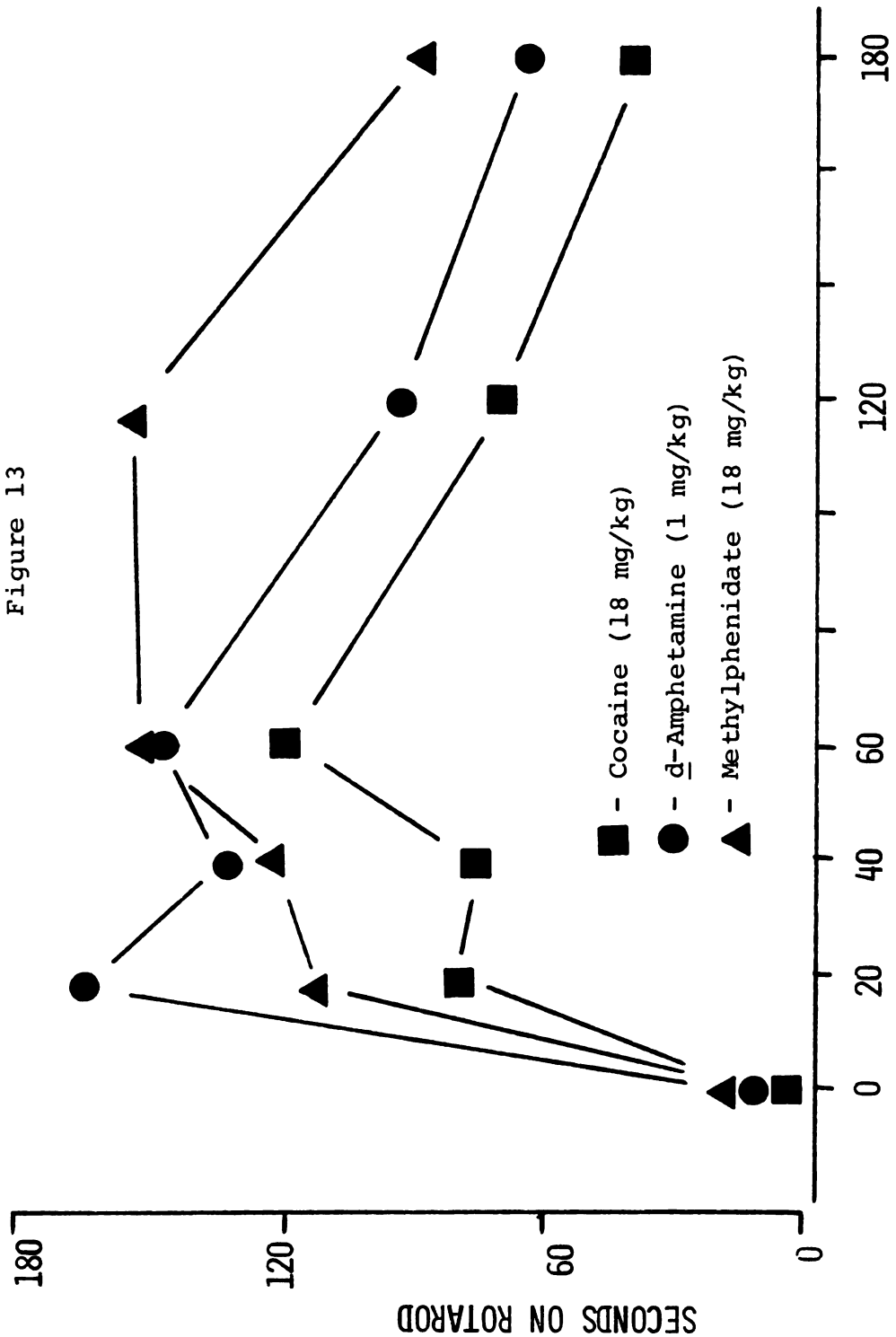

□ - Methylphenidate(18 mg/kg)
● - d-Amphetamine(1 mg/kg)
△ - Cocaine(18 mg/kg)
SECONDS ON ROTAROD
50
25
0
0
60
120
180
TIME (MINUTES)

Figure 12

Figure 13. Time course for long-term antagonism of reserpine depression of rotarod performance following d-amphetamine (1 mg/kg), methylphenidate (18 mg/kg) and cocaine (18 mg/kg). Results are expressed as the mean time each group remained on the rotarod (n=8). Testing protocol is described in the Methods section. All median scores obtained after CNS stimulant administration are significantly different from the control median score obtained just prior to treatment with stimulants. Significant differences are shown with an asterisk (multiple comparisons by nonparametric simultaneous test procedure of data within each drug group, $p < .05$).

Figure 13


SECONDS ON ROTAROD
180
120
60
0
0
20
40
60
120
180
TIME (MINUTES)
■ - Cocaine (18 mg/kg)
● - d-Amphetamine (1 mg/kg)
▲ - Methylphenidate (18 mg/kg)

Pirch et al., 1967; Rech, 1964). Only doses of 1.0 and 18 mg/kg of d-amphetamine and methylphenidate, respectively, were evaluated since these effects have previously been well characterized.

Cocaine, d-amphetamine and methylphenidate apparently exerted their central effects at noradrenergic, dopaminergic and serotonergic synapses (Glowinski, 1966; Smith, 1963; Moore, 1974; Rech, 1964; Rech et al., 1966; Hill et al., 1961; Vrbanac and Tilson, 1973; Vrbanac et al., 1975; Stolk and Rech, 1968; Usdin, 1974; Thut, 1974; Sigg, 1965; Scheckel et al., 1969; Taylor and Snyder, 1970; Iversen and Iversen, 1975). Proposed drug actions at these sites include blocking of neurotransmitter reuptake processes, release of neurotransmitters from their storage vesicles, direct presynaptic and postsynaptic receptor stimulation, and inhibition of MAO (Moore, 1971; Iversen and Iversen, 1975). The central actions of cocaine have usually been attributed to interference with neurotransmitter reuptake processes (Goodman and Gilman, 1975). Figures 12, 13 and 14 show cocaine to be a very effective antabonist of reserpine in this particular experimental situation, although less effective than d-amphetamine and methylphenidate acutely (i.e., 2 hours after reserpine). TCA are also potent blockers of reuptake processes for these neurotransmitters, and this effect is thought to be involved in the anti-reserpine activity of these agents. Another similarity between these drugs is that both the TCA and cocaine potentiate the stimulant and lethal effects of d-amphetamine.

Figure 14. Log-spaced dose-response for acute and long-term antagonism of reserpine depression of rotarod performance following various doses of cocaine. Results are expressed as the mean time each group remained on the rotarod (n=6). Testing protocol is described in the Methods section. Group median scores that are significantly greater than controls (reserpine alone) are shown with an asterisk (Mann-Whitney U test, $p<.05$).

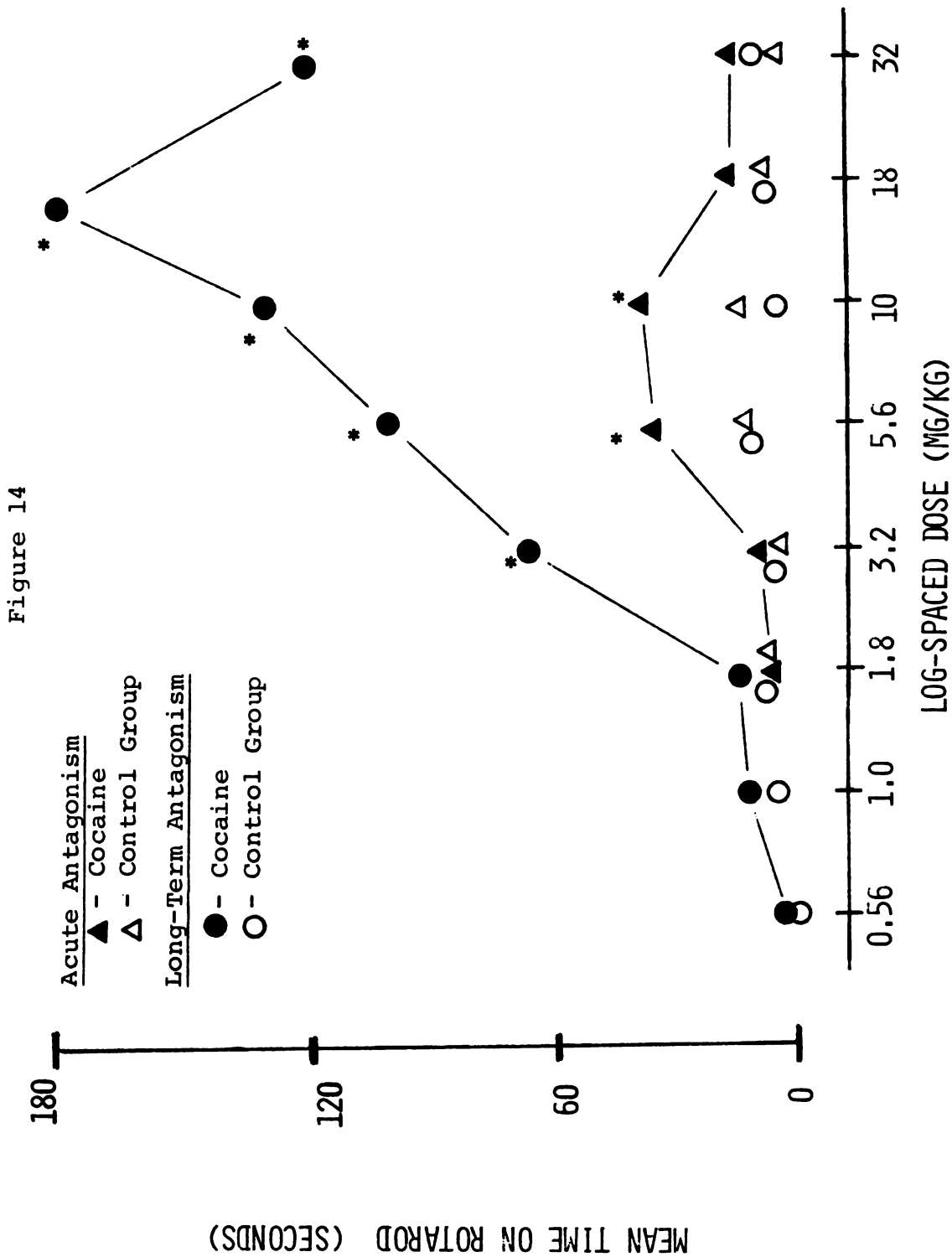

Acute Antagonism
▲ - Cocaine
△ - Control Group
Long-Term Antagonism
● - Cocaine
○ - Control Group
MEAN TIME ON ROTAROD (SECONDS)
180
120
60
0
0.56
1.0
1.8
3.2
5.6
10
18
32
LOG-SPACED DOSE (MG/KG)

Figure 14

The differences between these agents seen in this particular experimental situation apparently are related to other actions of the TCA and/or cocaine (i.e., anticholinergic, antihistaminic and antiserotonergic actions of the TCA, for example).

DISCUSSION

The results clearly demonstrate an inability of TCA or MAOI to antagonize the effect of reserpine in this particular experimental situation. d-Amphetamine clearly antagonized reserpine RR disruption and other behavioral impairments when administered 24 hours after the depressant in previous studies (Pirch et al., 1967; Rech, 1964). Comparing the RR score at 26 hours with those at 24 hours indicated that non of the antidepressant drugs were capable of antagonizing the RR impairments on the second day after reserpine, while the stimulants were very effective. Thus, none of these agents resembled d-amphetamine in interacting with reserpine. These observations are in agreement with the generally accepted profile of anti-reserpine activity seen for TCA, MAOI and d-A (Moore, 1971; Hill and Tedeshi, 1971; Iversen and Iversen, 1975).

Antidepressants have a much greater efficacy when given prior to reserpine treatment in attenuating the subsequent behavioral depression. TCA have been demonstrated to antagonize many of the peripheral effects of reserpine and tetrabenazine, but not the effects on operant behaviors (McKearney, 1968; Rech, 1974), at least in mammals. Thus, it is not so surprising that RR, a learned motor skill, was not reinstated by TCA to any degree following disruption by

reserpine. MAOI and caroxazone, however, were very effective in preventing the reserpine-induced RR impairment. These results show that caroxazone is functionally very different from TCA, but quite similar to MAOI in these interactions. They correlate with the reports by Moretti et al., (R.A. Carrano, personal communication, 1977) that caroxazone increased brain levels of catecholamines and 5-hydroxytryptamine and prevented their depletion by reserpine.

The time course of caroxazone's anti-reserpine effect differs markedly from that of the well-known MAOI over a wide range of doses, as depicted in Figures 9, 10 and 11. In a dose well above the threshold for short-term (4 hour) protection against reserpine, caroxazone's duration of action was no longer than 8 hours. Any very effective dose of phenelzine or isocarboxazid had a duration of anti-reserpine activity of at least 24 hours. This difference might be accounted for if caroxazone were a "reversible" MAOI, i.e., did not destroy MAO as a "hit-and-run" drug, as seems to apply for the classical MAOI (see Moore, 1971; Iversen and Iversen, 1975).

Many behavioral experimental paradigms result in data that is not normally distributed (nonparametric data). The data obtained for the RR-reserpine interactions shows an almost "all-or-none" characteristic. More than 95 % of all values obtained for reserpine controls (acetic acid vehicle) and for test drug controls (2.0 mg/kg reserpine treated subjects) occurred either between 1 and 5 seconds or were equal

to 180 seconds, the arbitrary "cut-off-point" (n>100). Traditionally, RR data has been expressed as the group mean score. Another common expression is the time to 50 % recovery of depressed RR performance (50 % of n). The data displayed in Figures 9, 10 and 11 show caroxazone to be approximately equipotent with phenelzine and slightly less potent than isocarboxazid. Although caroxazone is shown to exert a less consistent reversal of the reserpine-induced loss of RR performance, the maximal effect appears to be approximately the same as the maximal effect seen with the MAOI, generally speaking. However, caroxazone is seen to exert preventative effects that are quite different if the data is replotted in a quantal fashion (i.e., the traditional LDR). Figures 15 and 16 show the data contained in Figures 9, 10 and 11 expressed as the % of the subjects in each group showing complete protection from reserpine effects (i.e., % of subjects remaining on the RR for 180 seconds). The data is expressed as a % since not all groups contained the same number of subjects. Caroxazone is seen to exert anti-reserpine activity that is less effective than that afforded by the two MAOI. The effect is completely lost between the 6 and 8 hour readings, even for the highest doses of caroxazone. This is in sharp contrast to the preventative action of phenelzine and,to a slightly lesser degree, that of isocarboxazid. The four hour values seen for caroxazone cannot be compared to the LDR seen in Figure 8 since this latter curve was constructed using a larger number of subjects at doses > 3.2 mg/kg. If, however, the data for

Figure 15. Time course of complete prevention of reserpine depression of rotarod performance following various doses of caroxazone or phenelzine. Response is the % of subjects in each group remaining on the RR for the full 180 seconds, testing 4 hours after caroxazone or phenelzine and 2 hours after 2 mg/kg reserpine, with subsequent testing at 6, 8 and 10 hours after caroxazone or phenelzine. N=8 for the 3.2, 5.6 and 18 mg/kg caroxazone groups. For all other caroxazone groups n=7. For all phenelzine groups n=8. Data for repeated administration of caroxazone 4-hour maintenance dose (calculated from K_e=0.001925) after initial 32 mg/kg treatment is also shown (O).

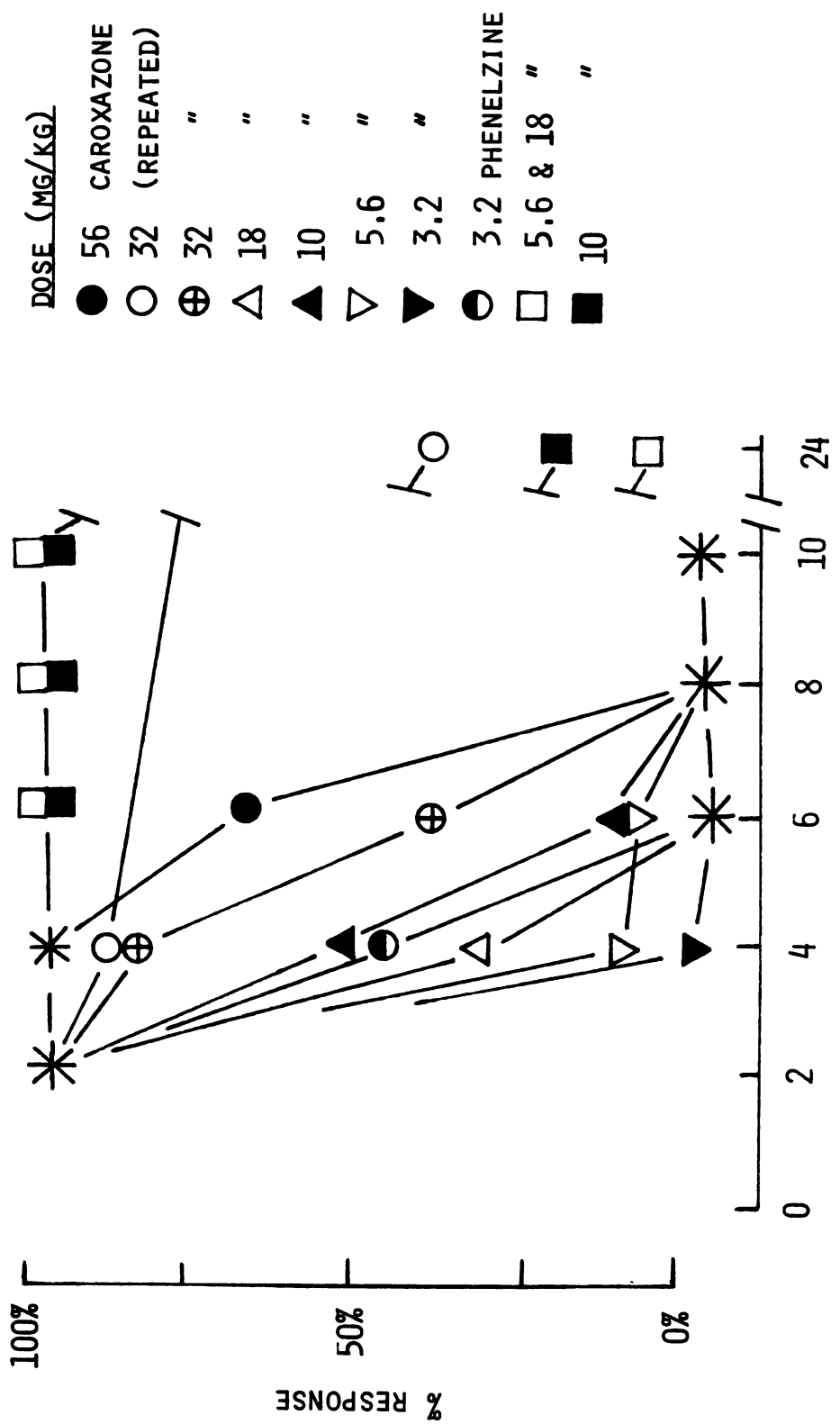

DOSE (MG/KG)
56 CAROXAZONE
32 (REPEATED)
32 "
18 "
10 "
5.6 "
3.2 "
3.2 PHENELZINE
5.6 & 18 "
10 "
100%
50%
0%
% RESPONSE
0
2
4
6
8
10
24
TIME (HOURS)

Figure 15

Figure 16. Time course of complete prevention of reserpine depression of rotarod performance following various doses of isocarboxazid. Response is the % of subjects in each group remaining on the RR for the full 180 seconds when tested 4 hours after isocarboxazid and 2 hours after 2 mg/kg of reserpine, with subsequent testing at 6, 8, 10 and 24 hours after isocarboxazid. Each group contained 8 subjects. Open-faced circles show results obtained upon repeated administration of 4-hour caroxazone maintenance doses (calculated for K_e=0.001925) after an initial 32 mg/kg treatment.

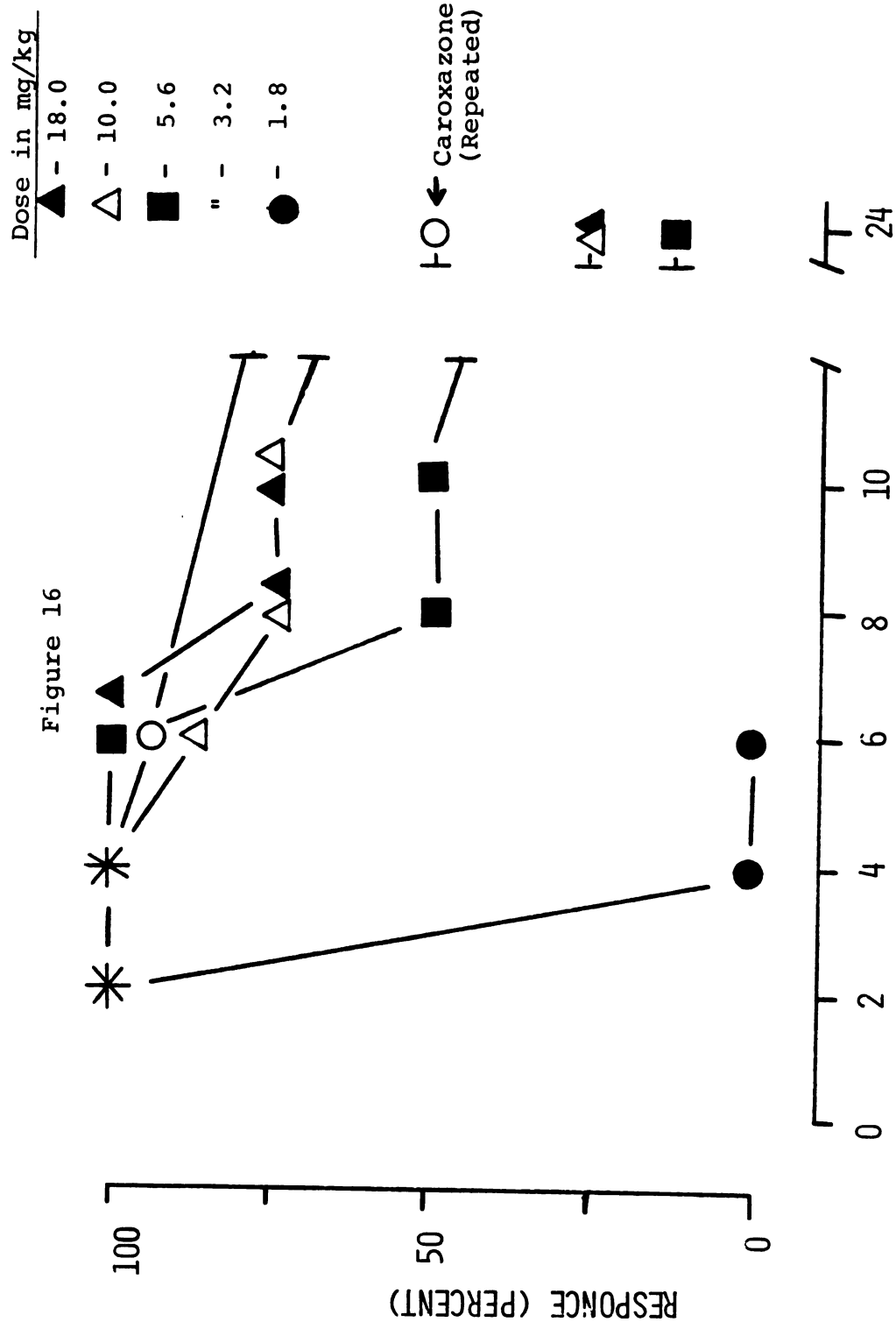

Dose in mg/kg
18.0
10.0
5.6
3.2
1.8
Caroxazone (Repeated)
RESPONCE (PERCENT)
100
50
0
0
2
4
6
8
10
24
TIME (HOURS)

Figure 16

caroxazone displayed in Figure 8 was replotted as the % of subjects performing to training criteria, then the dose of 5.6 mg/kg would be seen as approximately 50 % effective at 4 hours. Both Figures 15 and 16 show the data obtained for chronic (24 hour) administration of caroxazone. The effect differs dramatically not only from the single treatment caroxazone data, but paradoxically, shows caroxazone to exert greater protection at 24 hours than all doses of either MAOI.

Pharmacokinetic studies in man, dogs and rats have shown that the kinetics of elimination after repeated administration of caroxazone do not differ from single administration kinetics, nor does the pattern of tissue distribution change upon repeated administration (R.A. Carrano, personal communication). Thus, it seems very unlikely that caroxazone accumulated in these subjects. Besides, the total dose of caroxazone administered is only 98 mg/kg and not very different from the highest single dose used (56 mg/kg), on a log scale. The results obtained for repeated administration are consistent with the hypothesis that the anti-reserpine effects of caroxazone, and presumably its central effects in general, are completely reversible and are dependent upon blood levels of unbound caroxazone.

SECTION II

Prevention of αMethyltyrosine-induced Behavioral Depression: Comparison of Phenelzine and Caroxazone

SECTION II

INTRODUCTION

The previous study suggested that caroxazone is fundamentally different from the classical MAOI in the pattern of preventing the behavioral depressant effects of reserpine. This study attempts to further characterize the spectrum of behavioral effects of caroxazone. The amino acid α-methyltyrosine (αMT) is a reversible inhibitor of tyrosine hydroxylase and thus interferes with the synthesis of NE and DA (Spector et al., 1965). Because αMT enters the brain (via amino acid transport systems) it has been used extensively as a pharmacological tool to study the functions of central catecholaminergic neurons. Doses of αMT sufficient to maximally inhibit brain tyrosine hydroxylase activity, elicite a behavioral depression, with the onset and magnitude of this depression correlated with changes in catecholamine levels, and αMT-induced decreases in spontaneous motor activity, decrement of conditioned avoidance behavior and impaired performance of a learned motor skill have been reported (Moore, 1971; Moore and Rech, 1967; Rech et al., 1966). The effects of caroxazone on αMT-treated animals have not been reported. If administered to rats as a pretreatment, MAOI prevent αMT-induced behavioral depression as well as the αMT-induced reduction in NE and DA. TCA are without

effect in this test (Hill and Tedeshi, 1971; Iversen and Iversen, 1975; Moore, 1971; Moore and Rech, 1967; Rech, 1975). Based upon the results contained in Section I it was decided that phenelzine, which was nearly equipotent with caroxazone in preventing reserpine-induced depression of RR performance, would be compared with caroxazone for preventative effects against αMT-induced depression of the same learned motor skill. Repeated administration of αMT is necessary for this agent to consistently depress RR performance in rats to a level that is appropriate for evaluation of the preventative effects of a second drug. It was obvious from the results of Section I that repeated administration of caroxazone would be necessary and also appropriate.

METHODS

Subjects

All subjects used in this study were essentially as described previously in Methods, Section I.

Drugs

The drugs used in this study were caroxazone (free base), phenelzine (sulfate salt) and DL-α-methyltyrosine methylester (αMT; hydrochloride salt, Aldrich Chemical, Milwaukee, Wisconsin). Phenelzine was dissolved in 0.9 % NaCl (phenelzine vehicle). The concentration of phenelzine was adjusted for each dose such that the volume of 0.9 % NaCl injected at each treatment was always equal to 1.0 ml/kg body weight. αMT was dissolved in distilled water (D.W., αMT vehicle). The concentration of αMT was always 10 mg/ml of D.W. and the volume of injection for all subjects was 10 ml/kg body weight (to minimize renal toxicity resulting from precipitation of αMT in the renal tubules, Hook and Moore, 1969; Moore *et al.*, 1967). Caroxazone was suspended in 0.5 % methylcellulose (caroxazone vehicle). The concentration of caroxazone was adjusted for each dose such that the volume of 0.5 % methylcellulose injected at each treatment was always equal to 1.0 ml/kg body weight.

Training Procedure

The subjects were trained as described previously in the Methods portion of Section I.

Dosing and Testing Protocol

In the initial series of experiments subjects received a single i.p. injection of phenelzine in doses of either 0 (saline vehicle), 1.8, 5.6 or 18 mg/kg. There were 12 subjects receiving each dose of phenelzine or phenelzine vehicle. Half of the subjects in each of the four groups were treated 2, 5 and 8 hours later with 100 mg/kg αMT. The remaining subjects received αMT vehicle alone. Thus, there were a total of 8 treatment groups with 6 subjects in each group. Two hours following the last injection of αMT all subjects were tested for rotarod performance. The total time on the rotarod, up to 180 seconds, was recorded for each subject.

In the next series of experiments subjects were administered caroxazone in doses of 0 (0.5 % methylcellulose vehicle), 2, 4, 8, 10, 16, 32 and 64 mg/kg three hours before the first injection of αMT or αMT vehicle (total number of groups equal to 16 with 6 subjects per group). Caroxazone effects in the CNS are apparently completely reversible with elimination of the drug from the body (see Section I) and are thus directly related to the blood levels of the drug (R.A. Carrano, personal communication; Suchowsky et al., 1969a,b). Therefore, a maintenance dose of caroxazone was calculated (for K_e=0.001925 min^{-1}) and administered along with each of the three αMT treatments. Three hours

following the third injection of αMT (or αMT vehicle) plus caroxazone maintenance dose (or caroxazone vehicle) subjects were tested for rotarod performance. The total time on the rotarod up to 180 seconds was recorded for each subject.

Statistical Analysis

Tests for statistical significance were the Mann-Whitney U test for differences between independent samples and the nonparametric multiple comparisons by simultaneous test procedure, an *a posteriori* test of samples with equal measures based upon U, the Wilcoxin-Mann-Whitney statistic (Sokal and Rohlf, 1969).

RESULTS AND DISCUSSION

The results of the initial experiments with phenelzine are seen in Table 4. αMT treatment alone depressed rotarod performance to about the same extent seen in a previous study of αMT-induced depression of rotarod performance (Moore and Rech, 1967). MAOI (phenelzine) pretreatment almost completely prevented this effect, as expected. Doses of 5.6 and 18 mg/kg of phenelzine are seen to protect against the deficit while the 1.8 mg/kg dose was not effective (nonparametric simultaneous test procedure).

The results of the caroxazone experiments are seen in Table 5. αMT treatment alone significantly depressed rotarod performance ($p<.01$) to about the same extent as was seen in previous experiments. Doses of caroxazone in the range of 8-64 mg/kg were active in preventing the impaired rotarod behavior caused by αMT injections. All doses of caroxazone alone were seen to have no effect on rotarod performances.

Figure 17 displays log-spaced dose-response (LDR) curves for phenelzine and caroxazone effectiveness in preventing αMT-induced loss of rotarod performance. Response is plotted in the traditional manner as the % of the subjects responding (remaining on the rotarod for 180 seconds). The LDR curves show the drugs to be approximately equipotent in preventing αMT-induced behavioral depression. The minimum effective

TABLE 4

The effects of phenelzine and αMT given alone and in combination on rotarod performance

	Mean Time in Seconds on Rotarod (±S.E.M.)[1]			
	Pretreatment			
	0.9 % NaCl	Phenelzine sulfate in 0.9 % NaCl		
Dose of phenelzine→	0.0 mg/kg	1.8 mg/kg	5.6 mg/kg	18 mg/kg
αMT vehicle control group (10 ml/kg distilled water)→	174.3±5.7	154.7±25.3	180.0± 0.0	180.0± 0.0
αMT treated subjects (100 mg/kg x 3 {3})→	48.5±6.7	61.8±21.3	147.0±21.3	155.3±24.7

	Significant Differences in Group Median Scores[2]							
	αMT treated subjects				:	αMT-vehicle treated		
Dose of phenelzine (mg/kg)→	0.0	1.8	5.6	18	:	0.0	1.8	5.6	18

[1]Each value is the mean time in seconds on the rotarod up to 180 seconds. Each group contained 6 subjects. Refer to the text for treatment and testing protocol.

[2]Underscoring shows the results of making all possible comparisons (at $p<.05$ for all groups and $p<.01$ if only the αMT treated subjects are compared; simultaneous test procedure for non-parametric data using the Wilcoxin-Mann-Whitney U-statistic).

TABLE 5

The effects of caroxazone and αMT when given alone and in combination on rotarod performance

Caroxazone dose (mg/kg)[2]		Mean time on rotarod in seconds (± S.E.M.)[1]			
		Treatment group			
Initial	Maintenance	n	αMT vehicle controls	n	αMT (100 mg/kg x 3)
0.0	0.0	18	176.3± 3.7	18	58.8± 7.9
2.0	0.59	6	167.8±12.2	6	124.2± 8.5
4.0	1.17	6	180.0± 0.0	6	108.8±20.1
8.0	2.34	6	180.0± 0.0	6	171.8± 4.4
10.0	2.93	6	180.0± 0.0	6	175.2± 4.8
16.0	4.69	6	180.0± 0.0	6	151.8±28.2
32.0	9.38	6	180.0± 0.0	6	180.0± 0.0
64.0	18.8	6	180.0± 0.0	6	162.3±10.2

	Significant differences in group median scores[3]									
	MT treated					:	MT vehicle treated			
Dose of phenelzine (mg/kg)	0	2	4	8	32		0	2	4	8	32

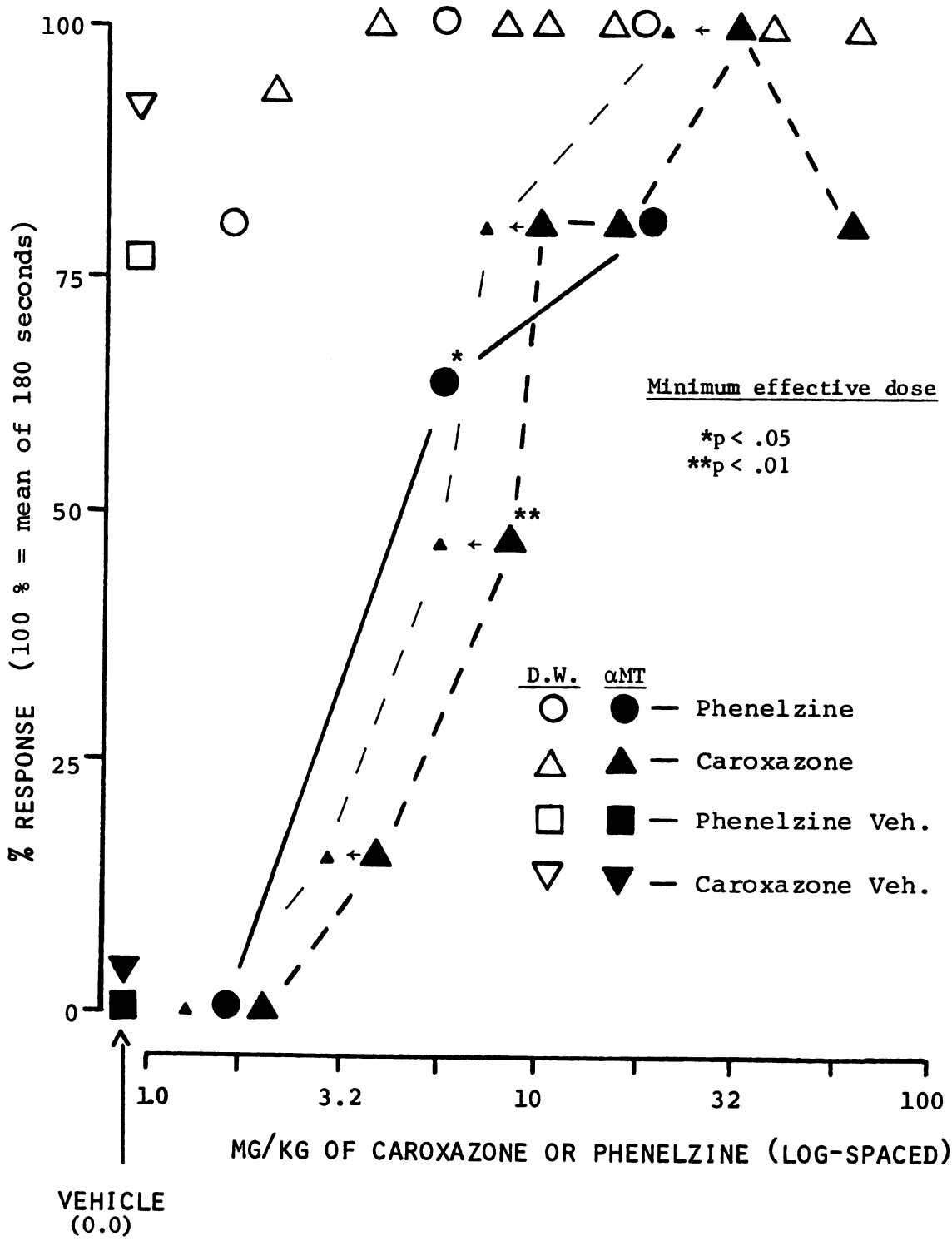
[1]Each value is the mean time on the rotarod up to 180 seconds ± S.E.M.

[2]Maintenance dose calculated for first order elimination ($T_{1/2}$ = 360 min).

[3]Multiple comparisons by simultaneous test procedures for nonparametric data ($p<.01$).

Figure 17. Log-spaced dose-response (LDR) curves for phenelzine and caroxazone efficacy in preventing αMT-induced loss of rotarod performance in previously trained rats. Response is the % of the subjects showing complete prevention of αMT effects (i.e., score is equal to control training criteria of 180 seconds). Open-faced characters are for paired MT vehicle controls (i.e., subjects treated with either phenelzine, caroxazone, phenelzine vehicle or caroxazone vehicle and also with distilled water, the αMT vehicle). Caroxazone and phenelzine are seen to be approximately equipotent in this test. The caroxazone LDR on the left takes into consideration the apparently completely reversible nature of the central effects of caroxazone (for K_e=0.001925).

Figure 17


100
75
50
25
0
% RESPONSE (100 % = mean of 180 seconds)
Minimum effective dose
*p < .05
**p < .01
D.W. αMT
Phenelzine
Caroxazone
Phenelzine Veh.
Caroxazone Veh.
1.0
3.2
10
32
100
MG/KG OF CAROXAZONE OR PHENELZINE (LOG-SPACED)
VEHICLE
(0.0)

doses (MED) are shown (*) to be 5.6 mg/kg and 8 mg/kg for phenelzine and caroxazone, respectively. The results of the previous study showed that caroxazone has anti-reserpine activity but that this activity is rapidly lost in a few hours as caroxazone blood levels fall. It is very likely that a similar situation would also exist for caroxazone's preventive effects on αMT-induced behavioral depression. The central actions of caroxazone are apparently completely reversible and thus the magnitude of a given drug effect is directly related to the time following administration of the drug. Phenelzine, on the other hand, exerts a stable level of "antidepressant" activity for many hours or even days after a single dose, presumable due to the irreversible nature of the MAO inhibition that outlasts the presence of the drug in the body. Thus, it was appropriate to calculate a "corrected" MED for caroxazone (for K_e=0.001925 min^{-1}). The corrected MED for caroxazone is 5.7 mg/kg.

SECTION III

Potentiation of L-DOPA-induced Behavioral Stimulation: Comparison of Phenelzine and Caroxazone

SECTION III

INTRODUCTION

L-DOPA (3,4-dihydroxyphenylalanine) penetrades the blood brain barrier freely despite the large hydrogen bonding capacity of this substituted amino acid. This is apparently due to its high affinity for the large neutral amino acid transport system (Oldendorf, 1974). Various effects are seen when L-DOPA is administered systemically to laboratory animals, including behavioral and electrophysiolocical changes. L-DOPA is an intermediate in the formation of dopamine (DA) and norepinephrine (NE) from phenylalanine (Blaschko et al., 1937; Gurin and Delluve, 1947) and L-DOPA by itself is considered to have little direct biological activity (Carlsson et al., 1957; Blaschko and Chrusciel, 1960; Smith and Dews, 1962). Since L-DOPA is the natural precursor of DA and NE, various effects seen following administration of L-DOPA have usually been attributed to enhanced activity of brain catecholamine pathways (Moore and Rech, 1967; Kadzielawa and Widy-Tyszkiewicz, 1970; Everett, 1961; Carlsson et al., 1957). Formation of excess amounts of DA and NE is apparently dose-dependent since the enzyme tyrosine hydroxylase is the rate-limiting step in the formation of NE and DA. The roles played in eliciting the various effects (behavioral and electrophysiological

observations) by NE and DA formed from exogenously-administered L-DOPA have been the subject of much debate (Chan and Webster, 1971; Ernst, 1969; Everett, 1968; Van Rossum et al., 1964; Rech et al., 1968; Taylor and Snyder, 1970; Rech and Thut, 1976). Other investigators have indicated that release of 5-hydroxytryptamine (5-HT) from central stores or the formation of active metabolites such as 3-O-methyl-dopa may contribute as well (Ng et al., 1970; Neuburg and Thut, 1974; Scheckel et al., 1969).

The DOPA-potentiation test devised by Everett (1967) and Everett et al. (1964) was originally intended to screen for non-MAOI antidepressant activity. Subjects were pretreated with a MAOI (pargyline) and subsequently administered a large dose of L-DOPA along with the compound being evaluated and the combination was compared with MAOI and L-DOPA along. The ability for test drugs to elicit peripheral adrenergic signs (sympathetic stimulation: salivation, piloerection, etc.) and central adrenergic activity (irritability, increased spontaneous motor activity, etc.) was the measure for antidepressant activity. This procedure was subsequently modified to screen for MAOI-type CNS activity (Rech and Thut, 1976; Thut and Rech, 1972). When relatively low doses of L-DOPA are combined with a MAOI (with peripheral decarboxylase activity having been inhibited by pretreatment with a third drug),the resultant effect on animal behavior is a very dramatic dose-dependent (doses of the MAOI) stimulation of spontaneous motor activity (along with the other signs indicative of general stimulation of the

catecholaminergic systems in the CNS). The procedure employed in this particular study is essentially the same as the previously reported, except that the test compound (phenelzine or caroxazone) was administered along with L-DOPA, instead of before L-DOPA treatment (Rech and Thut, 1976; Thut and Rech, 1972). The following study compares caroxazone with phenelzine for effectiveness and potency to potentiate the psychomotor stimulant and toxic effects of concomitant administration of L-DOPA and a peripheral decarboxylase inhibitor. The results contained in this study have been submitted for journal publication (Vrbanac et al., 1978c).

METHODS

Subjects

Female Sprague-Dawley rats, 200-250 g, and male Sprague-Dawley rats, 300-500 g (Spartan Farms, Haslett, Michigan) served as experimental subjects. Experiments measuring drug-induced alterations in spontaneous locomotor activity used only females as subjects. Males were used in experiments comparing phenelzine and caroxazone for potency to elicit a lethal effect to the combination of 32 mg/kg L-DOPA and 75 mg/kg HMD (see drugs below). Care of subjects was described earlier (Methods portion of Section I).

Drugs

The following drugs were used in this study: phenelzine (sulfate salt), L-dihydroxyphenylalanine (L-DOPA; Nutritional Biochemicals Corporation, Cleveland, Ohio), β-)3,4-dihydroxyphenyl)-α-hydrazine-α-methyl-DL-proprionic acid (HMD; Merck, Sharp and Dohme Research Laboratories, West Point, Pa.), caroxazone (free base), desipramine (hydrochloride salt) and amitriptyline. Phenelzine was dissolved in 0.9 % NaCl vehicle. All other drugs were suspended in 0.5 % methyl cellulose. Drugs were always administered i.p. in a volume of 1.0 ml/kg (first injection)

and 2.0 ml/kg (second injection).

Dosing and Testing Protocol

All subjects were pretreated with HMD, 75 mg/kg, to block peripheral decarboxylation of L-DOPA (HMD inhibits the enzyme L-aromatic amino acid decarboxylase and does not pass the blood brain barrier to any significant degree at this dose). Thirty minutes following HMD treatment half of the subjects received L-DOPA (32 mg/kg) plus either phenelzine or caroxazone (drug group) and the other half received L-DOPA or L-DOPA vehicle thirty minutes following HMD pretreatment. Each drug group (n=8) was paired with a control group (n=8) for all doses of phenelzine and caroxazone administered. One hour following the second injection individual subjects were placed in plastic animal cages (31 cm x 36.5 cm x 17 cm, l x w x h). Each cage was placed upon an electromagnetic motor activity apparatus (two Stoelting Electronic Activity Monitors). Counts were totaled for 15 minutes. Motor activity was also determined for 15-minute periods starting at two and three hours after the second injection. For each group of control subjects or drugged subjects half of the animals were recorded on each counter. Counts were always determined using the same counter at 1, 2 and 3 hours for any individual. The two counters were previously calibrated to give approximately the same number of counts for any given level of animal activity. Pairing of control subjects with drug subjects eliminated day-to-day variability as a factor since only results obtained on any

particular day were compared statistically. Testing for motor activity occurred only between 1300 and 1700 hours. Each subject was used only once. Doses of phenelzine used were 1.8, 3.2, 5.6, 10 and 18 mg/kg; caroxazone doses were 10, 18, 32, 56 and 100 mg/kg.

Statistical Analysis

The Mann-Whitney U test for differences between independent samples and the Wilcoxin ranked-sums test were used to determine significant differences ($p<.05$) between control and drug group median scores (both tests gave identical results). Comparisons between control (n=8) and drug groups (n=8) were made for each of the three sets of data (at 1, 2 and 3 hours) and for the three periods as accumulative motor activity counts.

RESULTS

The results of the motor activity experiments are seen in Figures 18 and 19. The control data comparing HMD plus L-DOPA with HMD plus vehicle is not shown but was found to be not significantly different or show any trend toward a difference ($p<.10$). Figure 18 shows the one, two and three hour counts obtained after various doses of phenelzine were combined with 32 mg/kg of L-DOPA, with peripheral decarboxylase activity inhibited by HMD (n=8). The control mean (0.0 dose) was calculated using control group counts for all 5 doses of phenelzine (i.e., n=40). Significantly different drug and control group medians occurred at doses of phenelzine of 3.2 mg/kg or greater. The 3.2 mg/kg data was not quite significant at 3 hours and just significant at 1 and 2 hours ($.025\ p<.05$). The 5.6 mg/kg dose clearly potentiated L-DOPA, the effect being quite dramatic ($p<.01$). The lowest dose, 1.8 mg/kg, did not significantly increase the median motor activity compared to vehicle control. The 1.8 mg/kg dose produced a slight but not significant interaction ($.05\ p<.10$). Thus, 3.2 mg/kg is a threshold or minimally effective dose of phenelzine in this particular experimental situation. The maximally effective dose was clearly 10 mg/kg. Phenelzine at 18 mg/kg produced serious toxicity. Two subjects died before the one hour reading and had

Figure 18. Phenelzine interaction with L-DOPA following HMD pretreatment. Animals were treated with 75 mg/kg HMD and 30 minutes later with 32 mg/kg of L-DOPA and 0.0, 1.8, 3.2, 5.6, 10 or 18 mg/kg of phenelzine. The average counts for 8 subjects is shown for each dose at 1, 2 and 3 hours after the last injection. The control average counts displayed are for all 40 control subjects. Each subject treated with phenelzine was paired with one control subject. Statistical significance was determined by the Mann-Whitney U test. A significant difference occurred at doses marked with an asterisk.

Figure 18

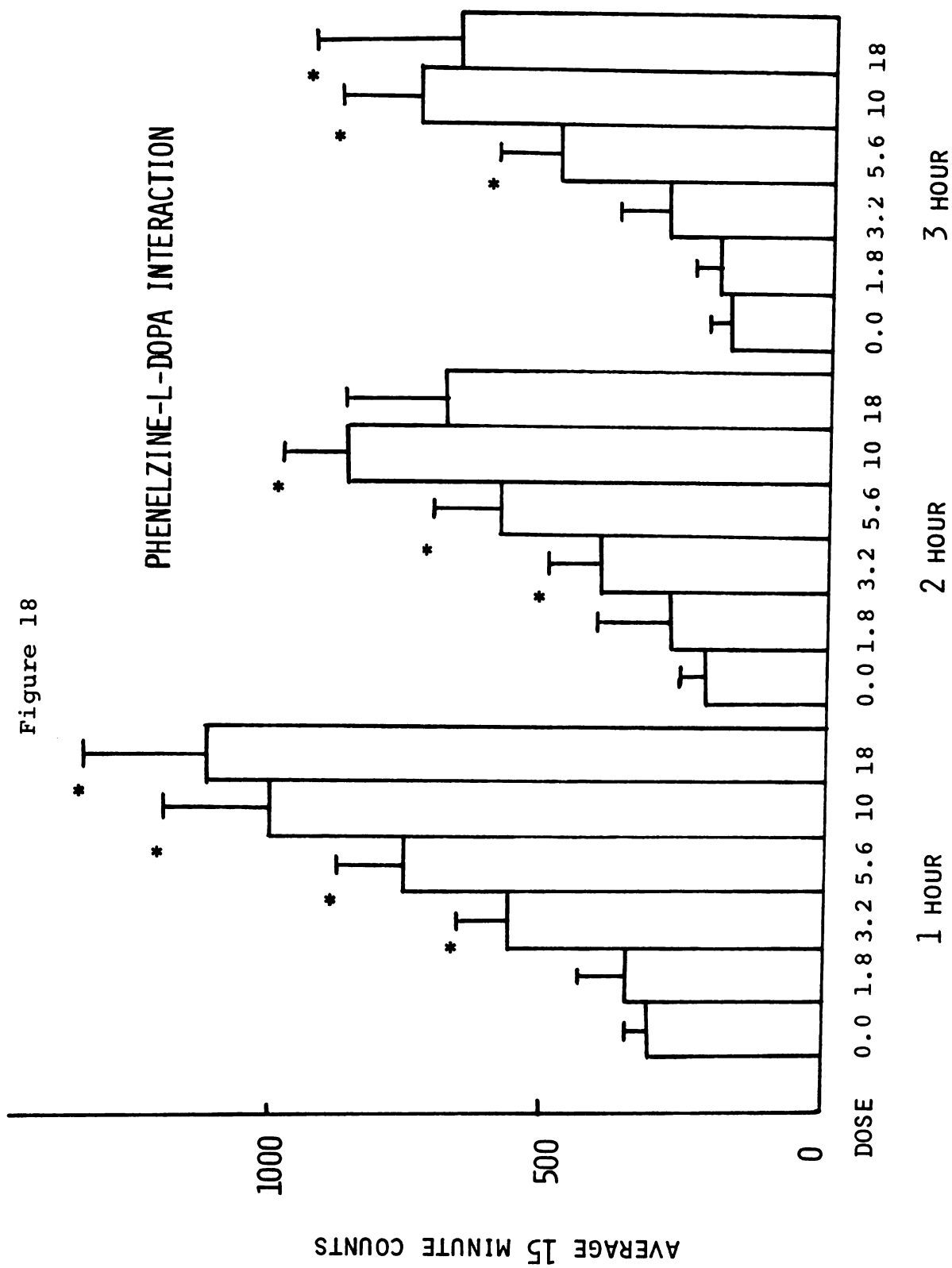
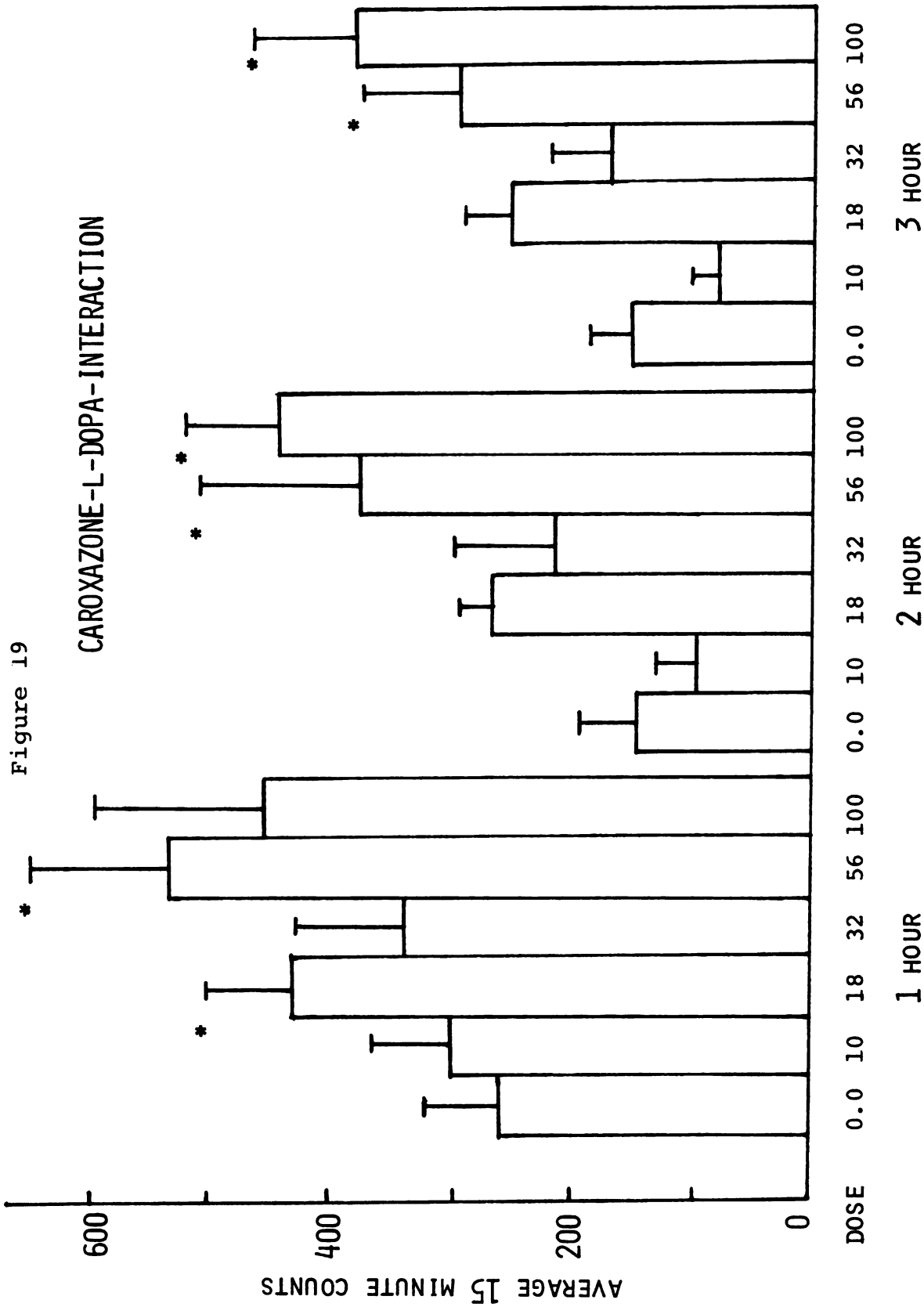

PHENELZINE-L-DOPA INTERACTION
AVERAGE 15 MINUTE COUNTS
1000
500
0
DOSE 0.0 1.8 3.2 5.6 10 18 0.0 1.8 3.2 5.6 10 18 0.0 1.8 3.2 5.6 10 18
1 HOUR
2 HOUR
3 HOUR

Figure 19. Caroxazone interaction with L-DOPA following HMD pretreatment. Animals were treated with 75 mg/kg HMD and 30 minutes later with 32 mg/kg L-DOPA and 0.0, 10, 18, 32, 56 or 100 mg/kg of caroxazone. The average counts for 8 subjects is shown for each dose as measured at 1, 2 and 3 hours after the last injection. The control average counts displayed are for all 40 control subjects. Each subject treated with caroxazone was paired with one control subject. Statistical significance was determined by the Mann-Whitney U test as described in the Methods section ($p < .05$). Significant differences occured at doses indicated (*).

Figure 19


CAROXAZONE-L-DOPA-INTERACTION
AVERAGE 15 MINUTE COUNTS
600
400
200
0
DOSE
0.0 10 18 32 56 100
1 HOUR
0.0 10 18 32 56 100
2 HOUR
0.0 10 18 32 56 100
3 HOUR
*

to be replaced. The other six subjects all lived but showed an overt behavior that was not consistent with the behavior seen with doses of 5.6 and 10 mg/kg. Instead of showing hyperreactivity to their environment, most of these subjects were relatively unresponsive to all types of environmental stimuli. Behavior was similar to the extreme stimulation seen shortly after a very high (lethal) dose of d-amphetamine in rats.

The caroxazone-L-DOPA interaction data is seen in Figure 19. The one obvious difference is an approximate 10-fold shift in the threshold dose needed for a significant, or almost significant increase in motor activity as compared to the dose-response pattern with phenelzine. Figure 20 plots the percent increase in accumulative counts (counts for 1, 2 and 3 hour periods are summed) above control as a function of log dose. The potency to produce an effect is not the only difference noted. The maximal effects (maximal stimulation of motor activity counts) were also quite different. Caroxazone maximal effect was only half that of the phenelzine response. Caroxazone by itself did not decrease spontaneous motor activity over controls at doses of 56 and 100 mg/kg in control. The results of the L-DOPA-interaction experiment were unexpected in the sense that 100 mg/kg of caroxazone did not appear to elicit a toxic interaction in this particular experimental situation, while 10 mg/kg of phenelzine appeared to be toxic and 18 mg/kg was lethal in 2 of 8 subjects tested. The dose-response curves for both phenelzine and caroxazone were extended to lethal effects.

Figure 20. Accumulative motor activity counts. Total counts over the three periods is expressed as % of paired control group mean accumulative counts and expressed as log-spaced dose-response curves for phenelzine and caroxazone treatments (n=8). Threshold doses for significant increases in accumulative counts were 1.8 mg/kg of phenelzine and 18 mg/kg of caroxazone. The maximal effect seen with phenelzine is two times greater than the maximal effect seen with caroxazone.

Figure 20

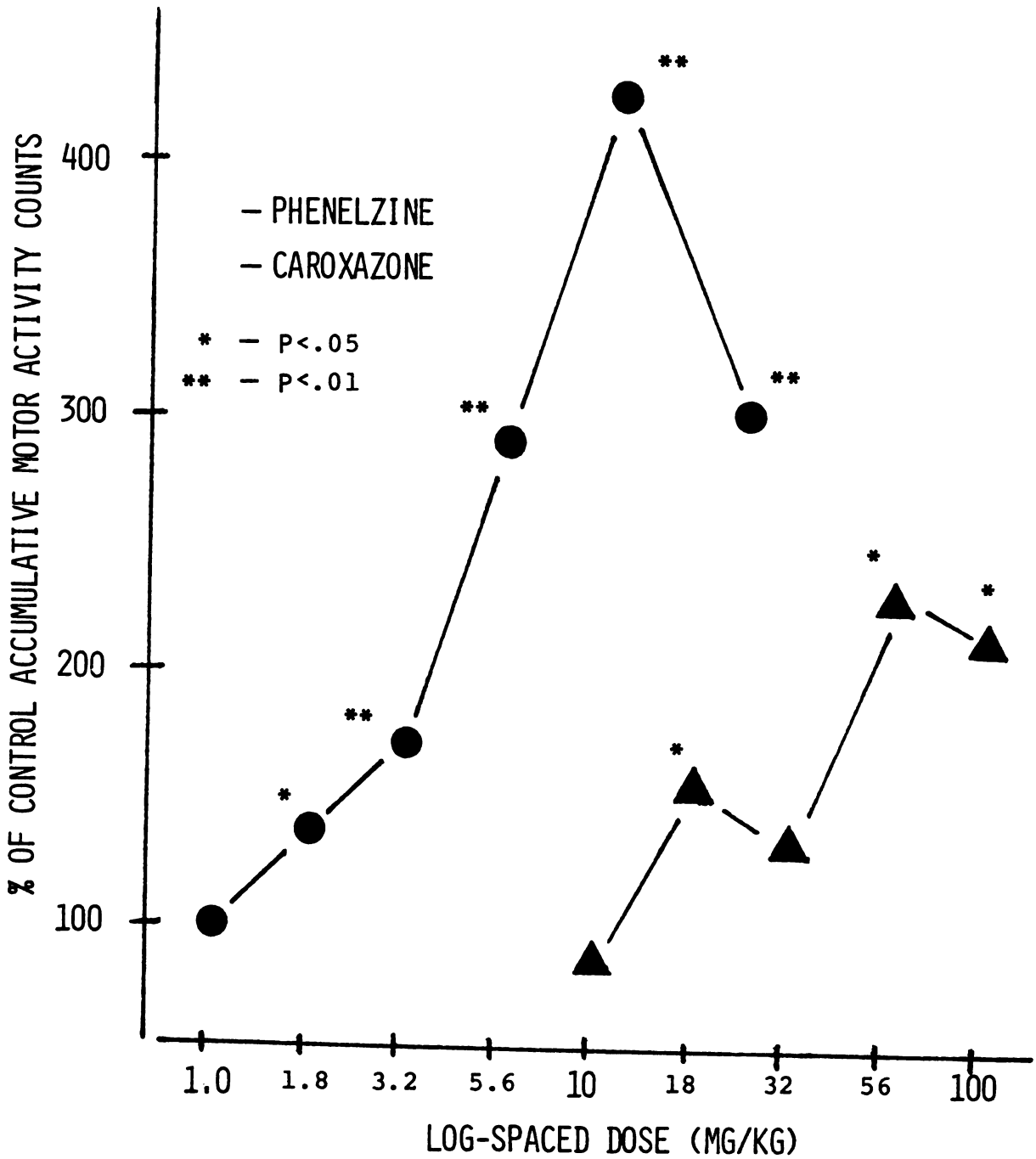

% OF CONTROL ACCUMULATIVE MOTOR ACTIVITY COUNTS
400
300
200
100
– PHENELZINE
– CAROXAZONE
* – P<.05
** – P<.01
1.0
1.8
3.2
5.6
10
18
32
56
100
LOG-SPACED DOSE (MG/KG)

TABLE 6

Caroxazone vs. phenelzine in potency to impart a lethal effect to the combination of 32 mg/kg L-DOPA and 75 mg/kg HMD

Test Drug Dose (mg/kg)	Number of Subjects Dead (24 hours)			
	Phenelzine		Caroxazone	
	Female	Male	Female	Male
10	0/8	---	0/8	---
18	2/8	---	0/8	---
32	---	0/8	0/8	---
56	---	3/8	0/8	---
100	---	8/8	0/8	---
180	---	4/4	---	---
320	---	---	0/8	0/8
560	---	---	2/4	0/8
MLD[1]	18	56	560	---[2]

[1] Minimum lethal dose observed for male and female subjects.

[2] The i.p. LD_{50} for caroxazone in rats has been reported to be 1532 mg/kg (R.A. Carrano, personal communication).

Because of limited supplies of caroxazone it was only possible to calculate a minimum lethal dose (MLD) using 8 subjects per dose. An LD_{100} was estimated for phenelzine. The results are seen in Table 6. Experimental protocol is unchanged from before. The obvious conclusion is that the phenelzine-L-DOPA interaction is dramatically more toxic than the caroxazone-L-DOPA interaction, at least over a 24-hour time span.

DISCUSSION

Spontaneous motor activity peak effects for caroxazone were found to be only half that for phenelzine. Doses of caroxazone greater than 100 mg/kg were not valid to include since depression of spontaneous motor activity was seen for caroxazone treated subjects at a dose of 180 mg/kg (4 subjects). The resultant behavioral effects of higher doses of caroxazone in combination with L-DOPA are therefore complex and would complicate the comparison with phenelzine data or with the lower doses of caroxazone. The quantitative differences between caroxazone and phenelzine measured in the motor activity experiments (potency and maximal effects) were no more dramatic than the qualitative differences observed in animal behavior and general appearance. These differences were so clear that caroxazone-DOPA and phenelzine-DOPA teated subjects were easily distinguished from one another on casual observation. The caroxazone combination resulted in far less overt hyperreactivity to environmental stimuli (such as hand clapping and handling), fewer and less pronounced signs of sympathetic stimulation and less fighting behavior. For example, subjects treated with 3.2 and 5.6 mg/kg of phenelzine subjectively appeared to show a more dramatic interaction with L-DOPA than subjects treated with 56 and 100 mg/kg caroxazone and L-DOPA. The relative

mildness of effects of the caroxazone-DOPA combination compared to the phenelzine-DOPA combination, even for the higher doses of caroxazone, is reflected in the MLD's of the two treatments (18 mg/kg vs. 560 mg/kg, for females). These findings add to the other evidence indicating basic pharmacodynamic differences between caroxazone and classical MAOI. Doses of caroxazone from 100 to 560 mg/kg should have been far more toxic than they were if caroxazone exerts central effects very similar to MAOI (i.e., inhibition of MAO activity *in vivo* towards biologically active amines derived from L-DOPA). Likewise, the signs of CNS stimulation at the lower doses should have resembled more the phenelzine-DOPA treated subjects.

The earlier data is worth discussing from another perspective. Reserpine exerts central depressant effects long after most of the administered dose has left the body. Persumably this action is due to depletion of 5-HT, NE and DA from nerve terminals by interference with processes necessary for neurotransmitter uptake and storage in synaptic vesicles. Exactly how this is accomplished is not known. However, it is reasonable to assume that this process involves binding of reserpine to receptors on or inside synaptic vesicles. The data obtained for phenelzine and isocarboxazid at 24 hours (Section I) indicates that $K_a >> K_d$ for this binding process and that the residual reserpine that remains after the bulk of the administered dose has been removed from the body is still biologically active. This has been suggested to be the case in the peripheral nervous system (Alpers and

Shore, 1967). Since the half-life for recovery of MAO activity following MAOI administration is in the order of many days, the loss of preventative effects at 24 hours indicates that some pharmacological action of the MAOI present at 10 hours has been lost, or diminished considerably, and that the loss of this drug action allows the residual reserpine to impair neuronal function, even though NE, DA and 5-HT stores have been protected. The existence of a small, functionally important, rapidly-recovering pool of MAO would fit into this hypothetical scenario. The presence of such a pool could also explain the lack of significant MAO inhibition (as measured in vitro) by caroxazone at lower anti-reserpine doses coupled with a spectrum of "antidepressant" activity that suggests a close relationship to the MAOI in bioassay procedures, since with this model only a small selective portion of central MAO activity need be inhibited for the expression of antidepressant effects. This latter proposal may also explain inconsistencies in the duration of functional effects of MAOI as compared with their duration of enzyme inhibition (Waldmeier and Maitre, 1976; Maitre and Waldmeier, 1976). These investigators showed that L-DOPA potentiation by MAOI, as well as the accumulation of dopamine and 3-methoxytyramine, was short-lived, less than 24 hours in half-life even with large doses of MAOI. Smaller doses of MAOI were observed to inhibit the enzyme (in vitro measurement) with a half-life of several days to over a week, in addition to provoking changes in homovanillic acid and

3,4-dihydroxyphenylacetic acid levels in striatum over this same time period.

Although the above speculations may seem attractive, it must be admitted that the relationship of the antidepressent activity of the MAOI to inhibition of this enzyme is still very tenuous.

SECTION IV

Comparison of Phenelzine, Desipramine, Amitriptyline and Caroxazone as Inhibitors of Cerebral Monoamine Oxidase: *In Vitro* Studies

SECTION IV

INTRODUCTION

The analytical techniques of gas-liquid chromatography (GC) and gas-liquid chromatography-mass spectrometry (GC/MS) mass-fragmentography have recently been very successfully applied to the qualitative and quantitative analysis of biogenic amine neurotransmitters and their metabolites. Most techniques developed from volatile electrophilic derivatives of these compounds. These electrophilic derivatives can be detected in picogram quantities by GC using electron capture detection (ECD). The GC/MS mass-fragmentography technique also involves the formation of a stable volatile derivative of the compound under analysis as well as a known amount of a deuterium-labeled internal standard. The relative intersities of appropriate mass fragments for the compound being analyzed and its deuterated derivative can be used for quantitation. A very efficient analytical approach that combines the use of GC and GC/MS systems involves the use of the GC/MS system to positively identify retention time values of electrophilic derivatives of the compound in question and appropriate internal standards of chemical similarity, and then the use of GC/ECD in routine quantitative analysis of biological samples. Various GC and GC/MS techniques for the qualitative and quantitative

detection of homovanillic acid (HVA, 3-methoxy-4-hydroxy-phenylacetic acid) (Sjoquist et al., 1973; Dziedzic and Gitlow, 1973; Gordon et al., 1974; Sjoquist and Anggard, 1972), 3-methoxy-4-hydroxyphenethylene glycol (MHPG) (Dziedzic and Gitlow, 1973; Dekirmenjian and Maas, 1974), 3,4-dihydroxy-phenylacetic acid (DOPAC) (Pearson and Sharman, 1974), normetanephrine (NM) (Narasimhachari, 1974; Lhuguenot and Maume, 1974; Abramson, 1974; Capella and Horning, 1966), vanillyl mandelic acid (VMA) (Gordon et al., 1974; Narasimhachari, 1974; Watson and Wilk, 1974), dopamine (Lhuguenot and Maume, 1974; Abramson, 1974; Capella and Horning, 1966; Kilts et al., 1976; Koslow et al., 1972), norepinephrine (Abramson, 1974; Capella and Horning, 1966; Koslow et al., 1972; Lhuguenot and Maume, 1974), 5-Hydroxyindole acetic acid (5-HIAA) (Watson and Wilk, 1974; Bertilsson et al., 1972), serotonin (5-HT) (Capella and Horning, 1966; Abramson, 1974; Cattabeni et al., 1972) and 3-methoxytyramine (3-methyxy-4-hydroxyphenyl ethylamine) (Capella and Horning, 1966; Kilts et al., 1976; Abramson, 1974) have been reported in the literature.

This section describes experiments designed to assess some in vitro neurochemical effects of caroxazone using GC/MS Mass-fragmentography. Caroxazone is compared with phenelzine, amitriptyline and desipramine for effectiveness to inhibit rat brain MAO in vitro, using 2,5,6-trideuterodopamine as substrate.

METHODS

MAO activity was determined by a slight variation of published procedures (Wurtman and Axelrod, 1964; Glowinski et al., 1966). Female Sprague-Dawley rats were decapitated and the brains removed. Forebrain was homogenized in 8 ml of ice-cold isotonic saline. To 1.0 ml of the homogenate were added 0.6 ml of pH 7.3 phosphate buffer and 8.4 ml of distilled water (D.W.) or D.W. containing various concentrations of caroxazone, desipramine, amitriptyline, or phenelzine (all salts were normalized as moles of free base). All tissues and drug solutions were fresh for each determination. The incubation medium was allowed to stabilize at 37°C for 15 minutes, with shaking, and then 100 µg of 3,4-dihydroxy-2,5,6-trideuterophenylethylamine hydrochloride (d_3-DA) was added as substrate. d_3-DA was prepared by acid exchange of the aromatic hydrogens in deuterium oxide acidified with deuterium chloride (Lindstroem et al., 1974; dopamine HCl was purchased from Aldrich Chemical Co., Milwaukee, Wisconsin). The chemical and isotopic purity of the d_3-DA used in this study was greater than 99 % (determined by GC/MS).

One hundred µl aliquots were placed in one dram vials at 0, 15 and 30 minutes following the addition of d_3-DA. Kinetic parameters for this particular experimental situation

were studied in an earlier experiment. Samples were taken every 5 minutes in the initial experiments (n=3) and every 15 minutes later on (n=5). The decline in substrate was linear up to 45 minutes. Linear regression analysis using 0-, 15-, 30- and 45-minute data points showed a significant correlation coefficient ($p<.01$). The correlation coefficient for regression analysis of the 0-, 15- and 30-minute data points was highly significant ($p<.001$). In all subsequent *in vitro* determinations of MAO activity samples were collected only at 0, 15 and 30 minutes. Enzyme activity was stopped by addition of 0.3 ml of methanol and 0.2 ml of 0.1 N HCl to each 100 µl sample of the incubation medium. Samples were heated in a water bath (60°C) under a stream of nitrogen until completely dry (10 to 20 minutes). To each sample, 30 µl ethylacetate and 20 µl pentafluoropropionic anhydride (PFPA) were added. Samples were dessicated and placed in a refrigerator until analysis could be performed. Standard curves were prepared each day by adding a fixed amount of dopamine HCl (264 pmol) and varying amounts of d_3-DA (52 to 1299 pmol). Each standard curve consisted of at least 7 points along with one blank (internal standard alone). The protein concentration in each incubation medium was determined by the method of Lowry *et al.* (1951). Enzyme activity was expressed as nanomoles d_3-DA metabolized/hour/mg protein and is shown in the test as a percentage of the total MAO activity inhibited. Control reaction velocity determined under these conditions was shown in an earlier experiment to be a close approximation of Vmax (determined by

Lineweaver-Burke plot of first-order decay data). The reaction velocity for 100 % inhibition was arbitrarily set as the rate of decay seen when 2 x 10^{-3} M phenelzine was present in the incubation medium.

Quantitation of DA in the samples was performed by multiple ion detection (MID, mass-fragmentography) using a Finnigan 3200E gas chromatography/mass spectrometry system interphased with a System Industries 150 data analysis unit. A 1.6 m x 2 mm (i.d.) silanized glass column packed with 3 % SP-2250 on 80.100 Supelcoport was used for separation. From 0.1 to 0.5 µl of each sample was injected into the system. Injection port temperature was 250°C and the column temperature was 155°C. The He carrier gas flow rate was 10 ml/min. Fragmentation was accomplished by electron impact at 70 eV and 0.5 mA. Ion pairs selected for monitoring of DA were m/e^{+}=431/428,284/281 and 268/265. This analytical technique has been described in detail previously along with analysis of the fragmentation pattern of the pentafluoropropionic anhydride derivative of DA by electron impact at 70 eV (Kilts et al., 1976). If the reader examines this reference the following corrections should be noted. With respect to the fragmentation pattern of the pentafluoropropionic anhydride derivative of 3-methoxytyramine, it was stated that m/e=296 results from loss of $OCOC_2F_5$ from the 4 position on the aromatic ring. The m/e=296 results primarily from cleavage between the α-carbon and the nitrogen. Also, for both DA and 3-MT, the α-carbon-nitrogen cleavage involves loss of one hydrogen from the α-carbon to the leaving group.

Figure 21. Electron impact mass spectra of 3-methoxytyramine, 3-methoxytyramine-d_3, dopamine and dopamine-d_3 pentafluoropropriony1 derivatives. See test for instrument conditions.

Figure 21

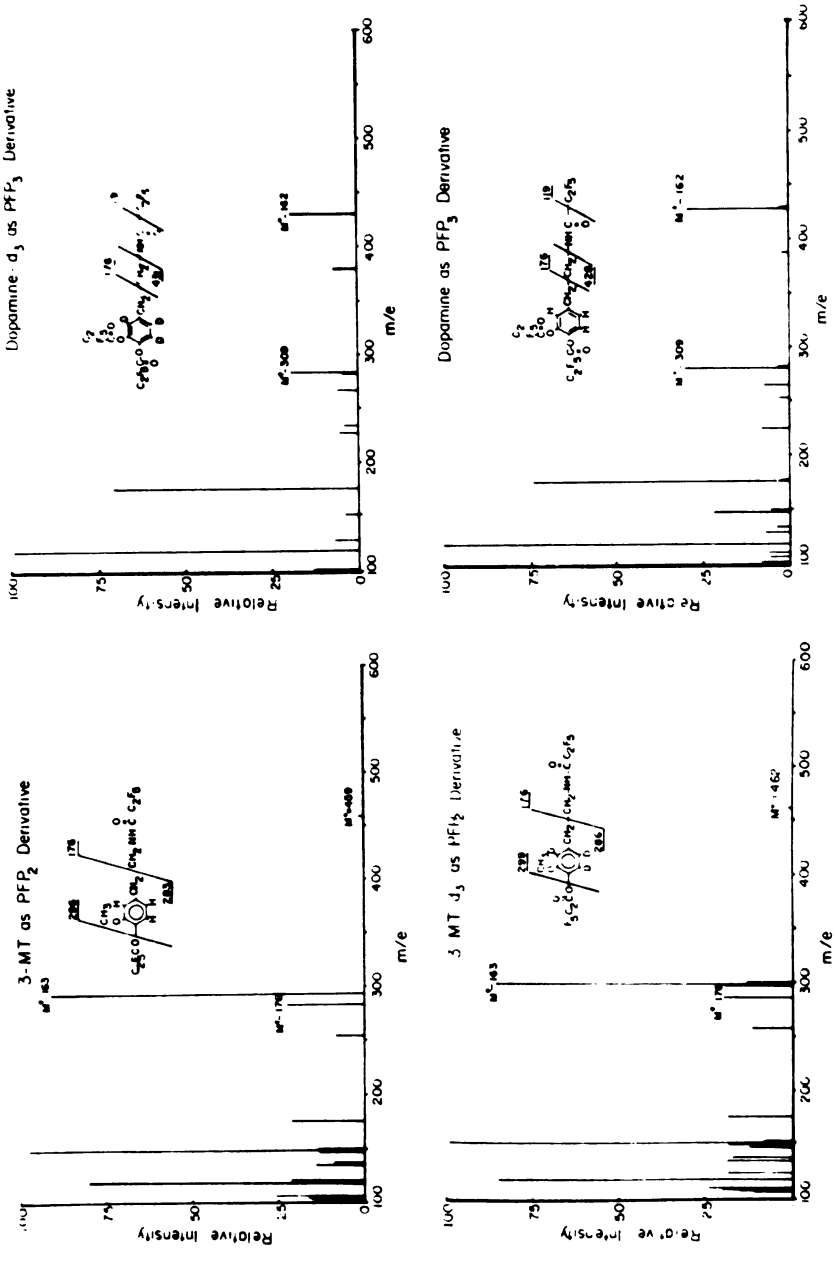

Dopamine-d3 as PFP3 Derivative
Dopamine as PFP3 Derivative
3-MT as PFP2 Derivative
3-MT-d3 as PFP2 Derivative
Relative Intensity
m/e

Figure 22. Electron impact mass spectra of a mixture of dopamine and d_3-dopamine, pentafluoropropionic anhydride derivative. Ordinate plots relative intensity and the abscissa plots m/e^+. Scan range covers $m/e^+ = 250$ to $m/e^+ = 450$.

Figure 22

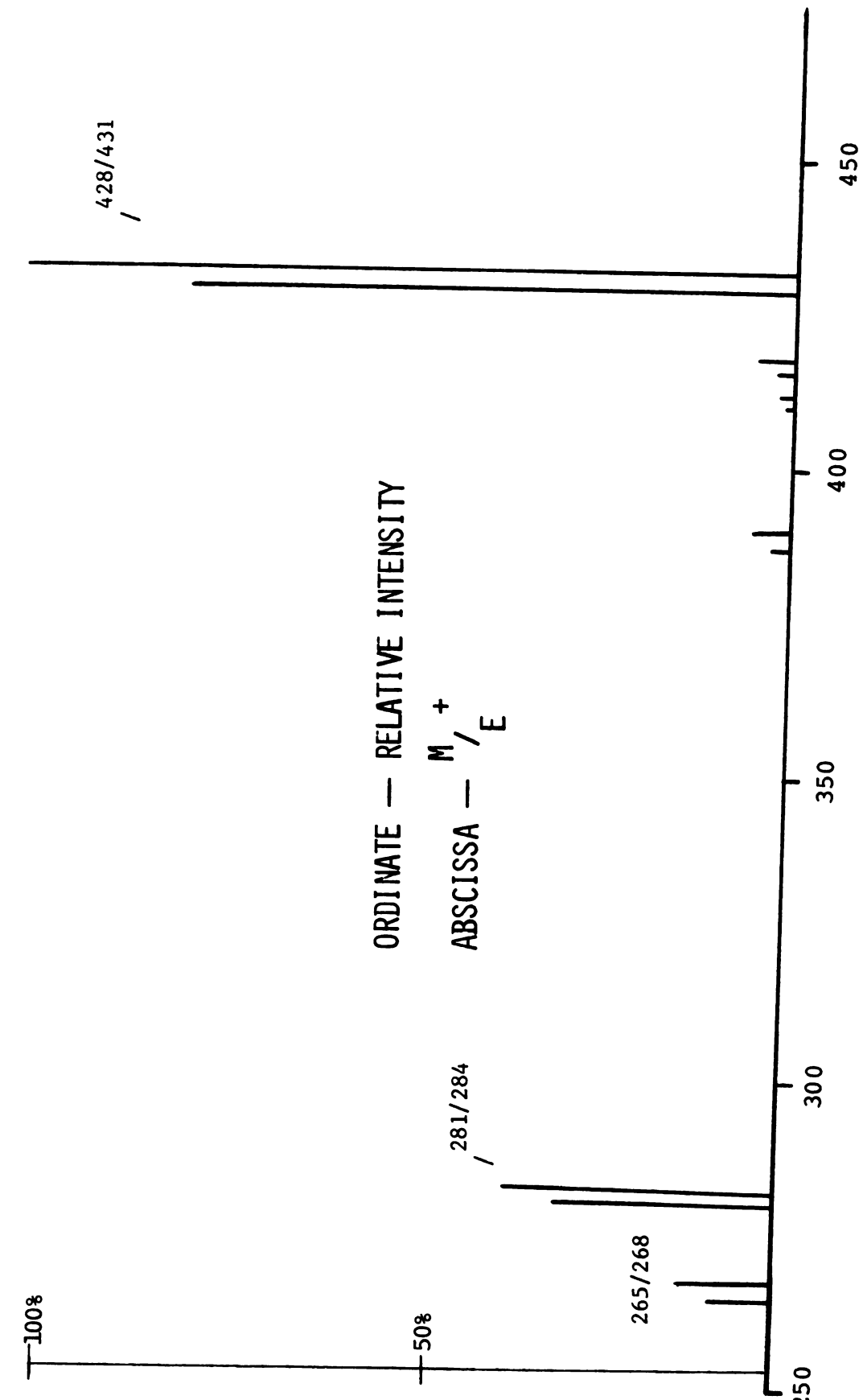

100%
50%
265/268
281/284
428/431
250
300
350
400
450
ORDINATE — RELATIVE INTENSITY
ABSCISSA — M+/E

Figure 23. Representative standard curve for d_3-dopamine quantitation by GC/MS mass fragmentography, 431/428 ion pair. The observed deuterium/protium (D/P) ion intensity ratio (ordinate) is plotted against the amount of d_3-dopamine injected into the GC/MS system (expressed as picograms of d_3-DA). The volume of injection was 1.0 µl.

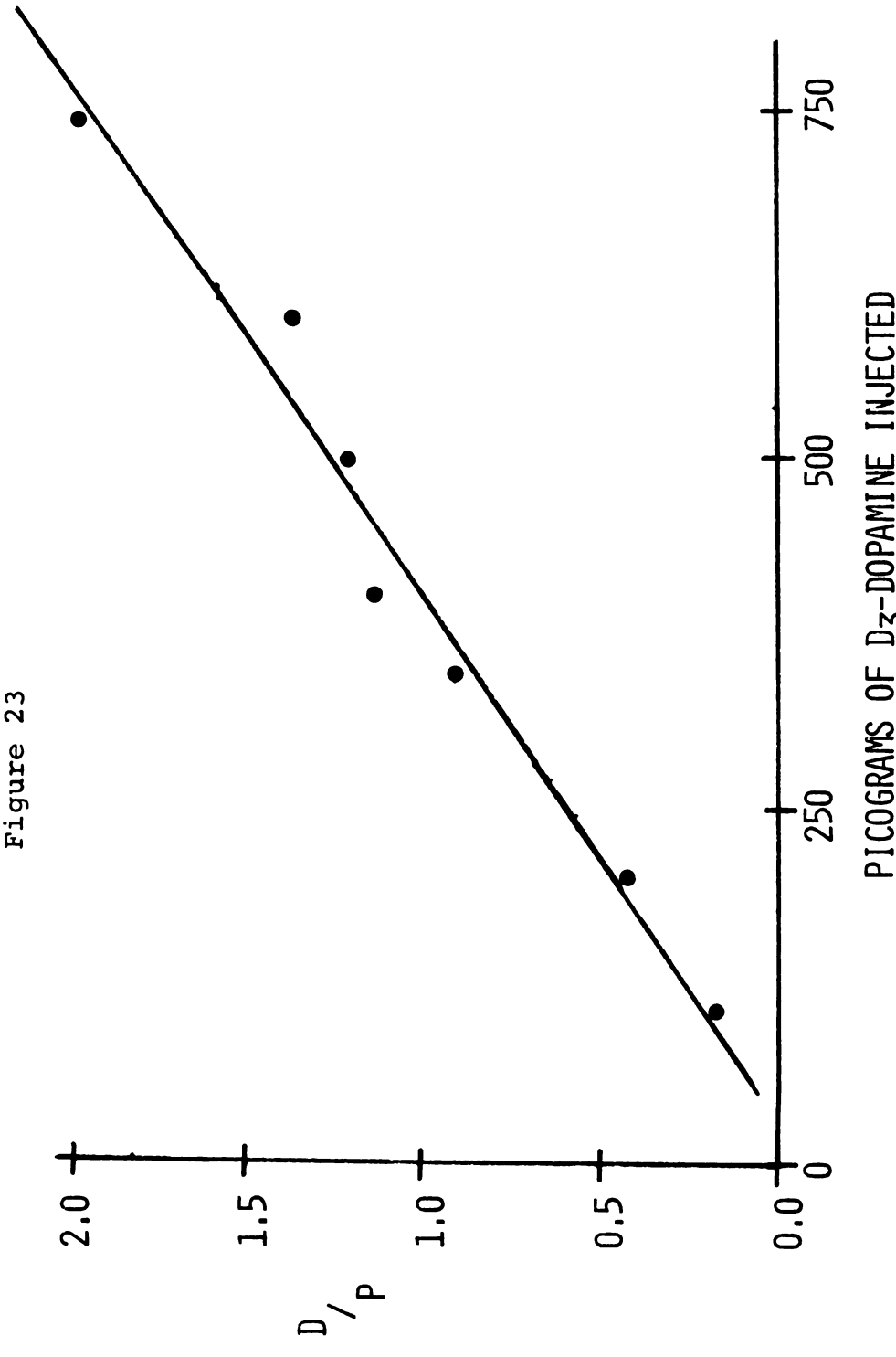

2.0
1.5
1.0
0.5
0.0
D/P
0
250
500
750
PICOGRAMS OF D3-DOPAMINE INJECTED

Figure 23

Figure 21 shows the fragmentation patterm for both compounds using the above conditions. Figure 22 shows the fragmentation pattern for a mixture of DA and d_3-DA (pentafluoropropionic anhydride derivative). Note that the scan range covers only m/e^+= 250 to m/e^+= 450. Figure 23 shows a representative standard curve for D/P = 431/428 ion intensities. Nanograms of d_3-DA present at 0, 15 and 30 minutes were used to calculate the reaction velocity (nanomoles/hr/mg protein). The reaction velocities seen for each drug concentration and for paired controls were compared using ANOVA and an appropriate test for significance (Student-Newman -Keul's procedure for multiple comparisons, $p<.05$).

RESULTS

The decline in d_3-DA over a 90-minute period following the addition of 100 µg of substrate in the control situation with samples taken every 15 minutes is displeyed in Figure 24 The regression line shown was calculated using the 0-, 15- and 30-minute data points (n=5, correlation coefficient significant at $p<.001$). The initial series of *in vitro* experiments determined rat brain MAO activity in the presence of 1×10^{-4} meq/ml phenelzine, desipramine, amitriptyline and caroxazone. The results are displayed in Table as nm d_3-DA/hr/mg protein (n=5). Refer to the Methods section for calculations. Data is also displayed as the % of control reaction velocity. Caroxazone and phenelzine are seen as very effective inhibitors of MAO activity, whereas amitriptyline showed a weak but significant effect and desipramine was without effect. The next series of experiments examined the rate of decay of d_3-DA over the initial 30 minutes in the presence of various concentrations of phenelzine, caroxazone, amitriptyline and desipramine. The mean of all control determinations was 45.36±3.57 (n=20) nm/hr/mg protein. In a separate experiment the addition of 2×10^{-3} M phenelzine resulted in a rate of decay of d_3-DA of 3.06±0.49 nm/hr/mg protein (n=6). These two values were used to construct Figure 25 (i.e., 0.0 % and 100 % inhibition of MAO activity).

Figure 24. Disappearance of 100 μg of d_3-DA over a 90-minute period. Each point represents the average of 8 determinations. Initial 30 minutes shows linear zero-order kinetics, and regression line is shown (correlation coefficient significant at $p<.001$). When 0, 15, 30 and 45 minute data is used, the correlation coefficient is still highly significant at $p<.01$. The initial 30 minutes approximates Vmax. Control reaction velocity was calculated to be approximately 45 nanomoles/hr/mg protein. Data shown is not corrected for protein differences (which were very small) between the 8 experiments.

Figure 24

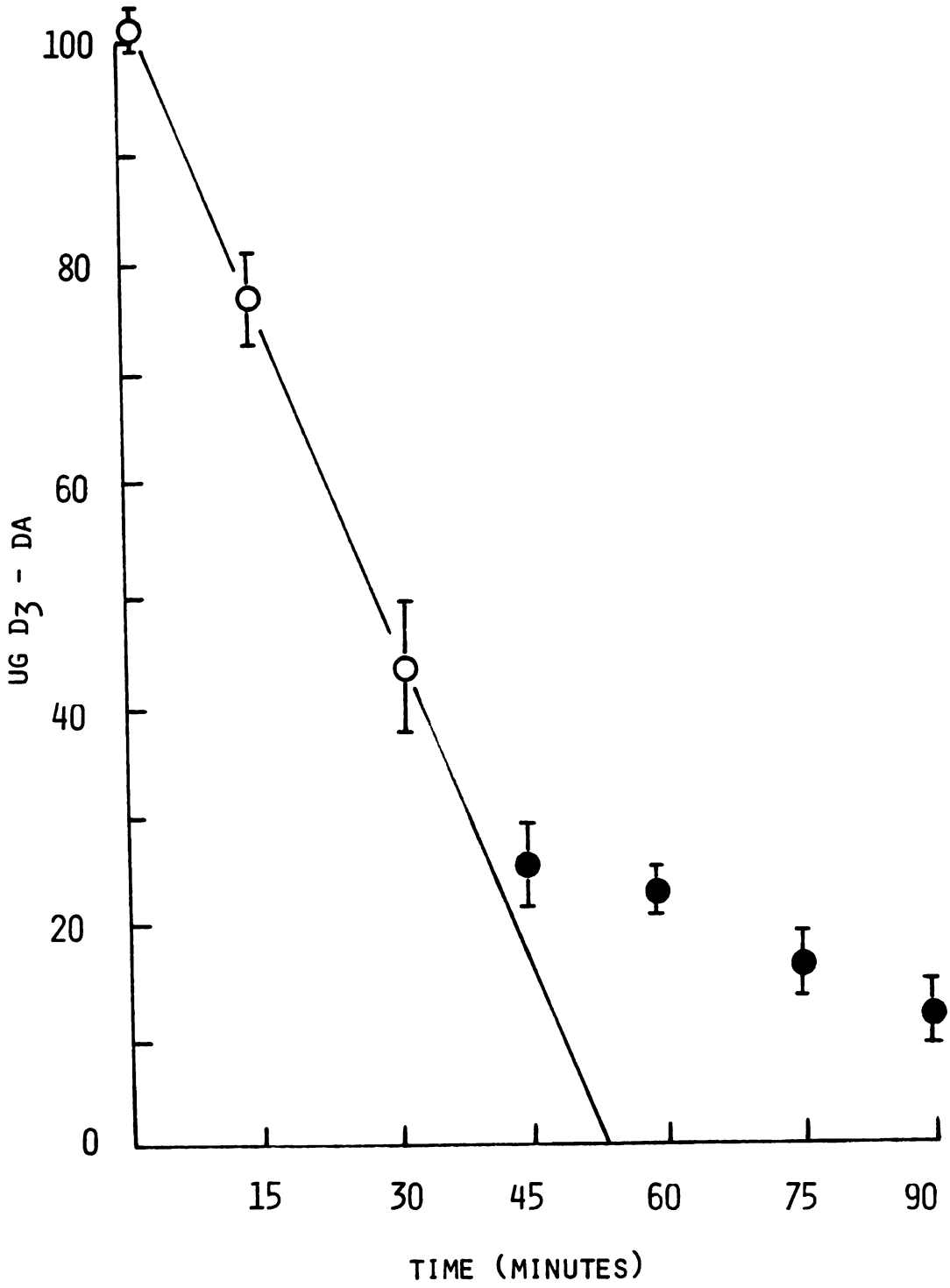


TABLE 7

In Vitro measurement of MAO activity toward d_3-DA as a substrate

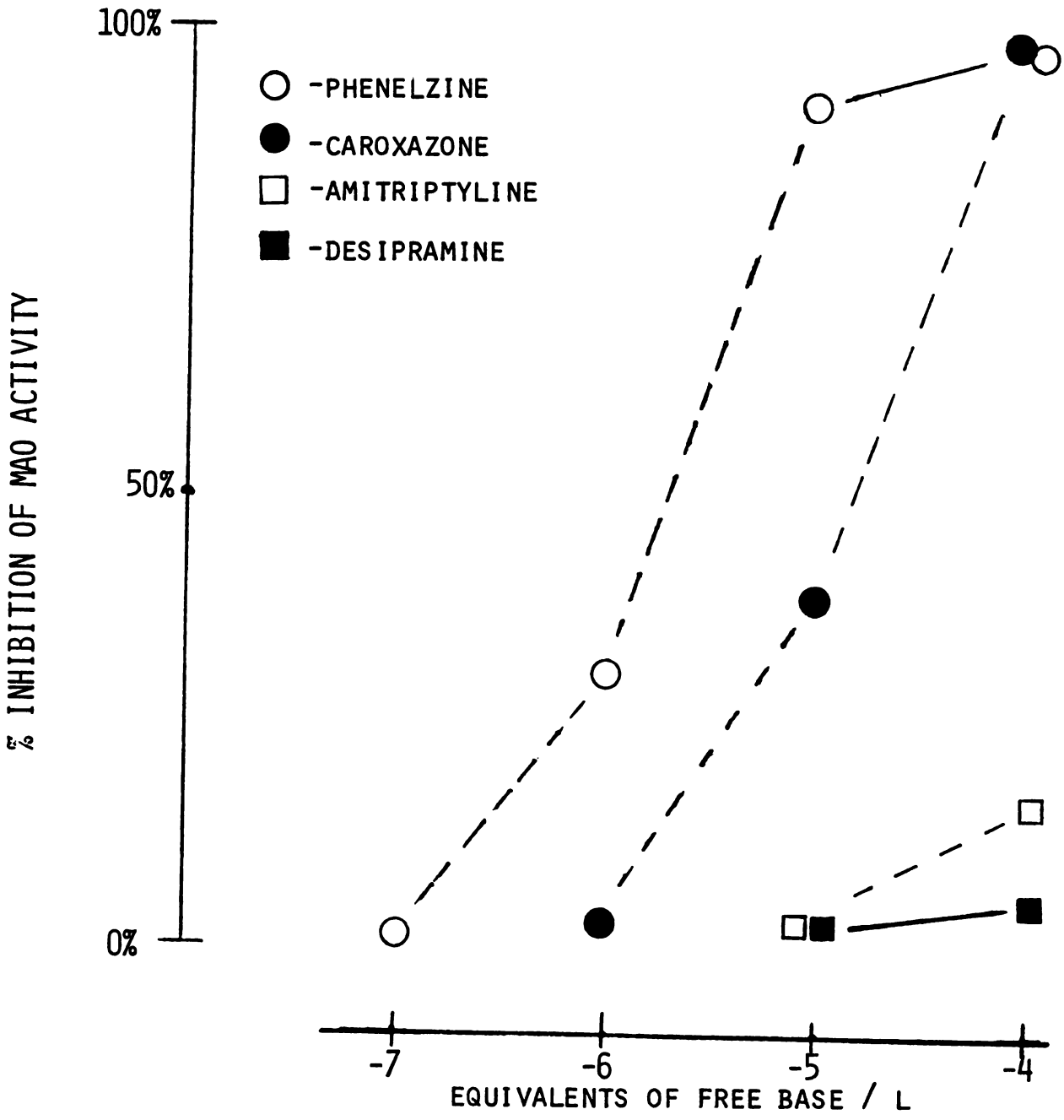
Drug added to incubation medium[1]	MAO activity[2] $\bar{X} \pm$ S.E.M.	N
1. None (controls)	46.24±4.43	5
2. Caroxazone	4.71±1.17	5
3. Phenelzine	4.22±0.37	5
4. Desipramine	45.73±2.56	5
5. Amitriptyline	38.38±2.98	5

[1]Concentration of inhibitor = 1×10^{-4} meq free base/ml for all drugs.

[2]Reaction velocity of DA decline with an initial concentration of 5.28×10^{-5} M d_3-DA, expressed as nanomoles/hr/mg of protein.

Figure 25. Estimation of the log dose-response curve for phenelzine, caroxazone, amitriptyline and desipramine *in vitro* inhibition of rat brain MAO activity with dopamine as substrate. Values used for 0 % and 100 % inhibition are 45.36±3.57 (n=20) and 3.06± 0.49 (n=5) nanomoles d_3-DA/hr/mg protein, respectively. Each point is the average (n=5) corrected reaction velocity expressed as a percent of these values. Drug concentrations are expressed as normality (meq free base/ml). Reaction velocities at different inhibitor concentrations were examined for significant differences (Student-Newman-Keul's procedure applied to phenelzine and caroxazone data and two-tailed t-test for amitriptyline and desipramine data). Points connected by broken lines are significantly different ($p<.01$) and points connected by solid lines are not different ($p<.05$).

Figure 25


100%
50%
0%
% INHIBITION OF MAO ACTIVITY
-PHENELZINE
-CAROXAZONE
-AMITRIPTYLINE
-DESIPRAMINE
-7
-6
-5
-4
EQUIVALENTS OF FREE BASE / L

Data is expressed as the % inhibition of MAO activity vs. $\log_{10}$ inhibitor concentration. Phenelzine and caroxazone at 10^{-4} M concentrations inhibited MAO activity towards DA maximally. Phenelzine at 10^{-5} N was almost maximally effective while 10^{-5} N caroxazone was slightly less than 50 % effective. Caroxazone was completely ineffective at 10^{-6} N while phenelzine was still somewhat effective. Estimated ED_{50}'s were approximately 2×10^{-5} N for caroxazone and 2×10^{-6} N for phenelzine. Thus, caroxazone is clearly less potent than phenelzine in inhibiting MAO *in vitro* when dopamine is the substrate. These results are in agreement with previous observations (Suchowsky *et al.*, 1969; R.A. Carrano, personal communication).

SECTION V

In Vivo Studies: Effect of Phenelzine and Caroxazone on the Concentration of Norepinephrine in Rat Hypothalamus

SECTION V

INTRODUCTION

The scientific literature is replete with data demonstrating the ability of both MAOI and TCA to increase the concentration of DA, NE and 5-HT in the mammalian central nervous system. Suchowsky et al. (1969b) reported that single of repeated doses (5 days) of caroxazone significantly increased the concentration of DA, NE and 5-HT in whole mouse brain. 5-Hydroxyindole-3-acetic acid (5-HIAA) was also reported to be increased by repeated administration of caroxazone, an observation that certainly does not fit with caroxazone exerting central activity through monoamine oxidase inhibition. The study described in this section extends this earlier work to more descrete brain nuclei. The effect of caroxazone and phenelzine on the concentration of NE in rat hypothalamus was determined using GC/MS mass fragmentography for NE quantitation.

METHODS

Subjects

Female Sprague-Dawley rats, 175-200 g were used. Specifics are as presented in Section I.

Drugs

Drugs used in these experiments have been detailed previously (Section I).

Dosing Protocol

Subjects were treated with various doses of phenelzine or caroxazone 2 hours prior to sacrifice. Phenelzine doses were 0.0, 1.8, 3.2, 5.6 and 10 mg/kg. Caroxazone doses were 0.0, 3.2, 10 and 100 mg/kg. Drug solutions were prepared as described previously.

Determination of NE by Mass Fragmentography

Following decapitation of subjects the brains were quickly removed and placed ventral side up in a petri dish on ice. A razor blade and spatula were used for instruments. An initial dorsal-ventral cut was made just at the optic chiasm. A second dorsal-ventral cut was made just posterior to the mammillary bodies. The section thus formed was turned with the anterior face up. An anterior-posterior cut is then just at the anterior commissure. The cortical tissue on both

sides of the hypothalamus was dissected away and other non-hypothalamic tissues removed. Approximately 30 mg of tissue was obtained from female Sprague-Dawley rats weighing 175 g or more. The tissue was immediately weighed and placed in homogenation tubes containing 1.0 ml ice cold 0.01 NE HCl and internal standard (50 ng β-d_1-α-d_2-NE HCl, Merck & Co., Isotopes). Tissues were homogenized, centrifuged (refrigerated) and the supernatant was extracted with 3 volumes of ethyl acetate. The aquious phase was dried in a water bath (50-60°C) under N_2. After drying samples were derivatized by adding 10 µl ethyl acetate and 40 µl pentafluoropropionic anhydride (Regis Chemical Co.) and heated for 15 minutes at 150°C. Standard curves were prepared (0-250 ng protonated compound plus 50 pg deuterated compound) and derivatized in the same manner. Quantitation of NE-4PFPA was performed by mass fragmentography. The standard curves were analyzed with linear regression and the sample NE levels calculated. Figure 26 shows the high end of mass spectra obtained for NE and d_3-NE. Ions monitored were 590/592, α-carbonnitrogen cleavage, and 577/578, α-β cleavage. Both ion pairs gave satisfactory standard curves. Values shown were calculated from the regression line obtained for the 577/578 ion pair and are not different from results obtianed using 590/592 regression line. Correlation coefficients for both plots were greater than .95. A representative standard curve is shown in Figure 27 (577/578 ion pair). The protium/deuterium ion intensity ratio is plotted against the amount of NE. The response is linear over the range shown.

Figure 26. TIM scans for NE and d_3-NE. Base peak is m/e^+ = 176 ($CH_2NH\text{-}OCOC_2F_5$). Relative abundance of 590 (592) is 60 %. Cleavage pattern is described in the methods section. Ion pairs 590/592 and 577/578 were used in mass fragmentographic analysis.

Figure 26

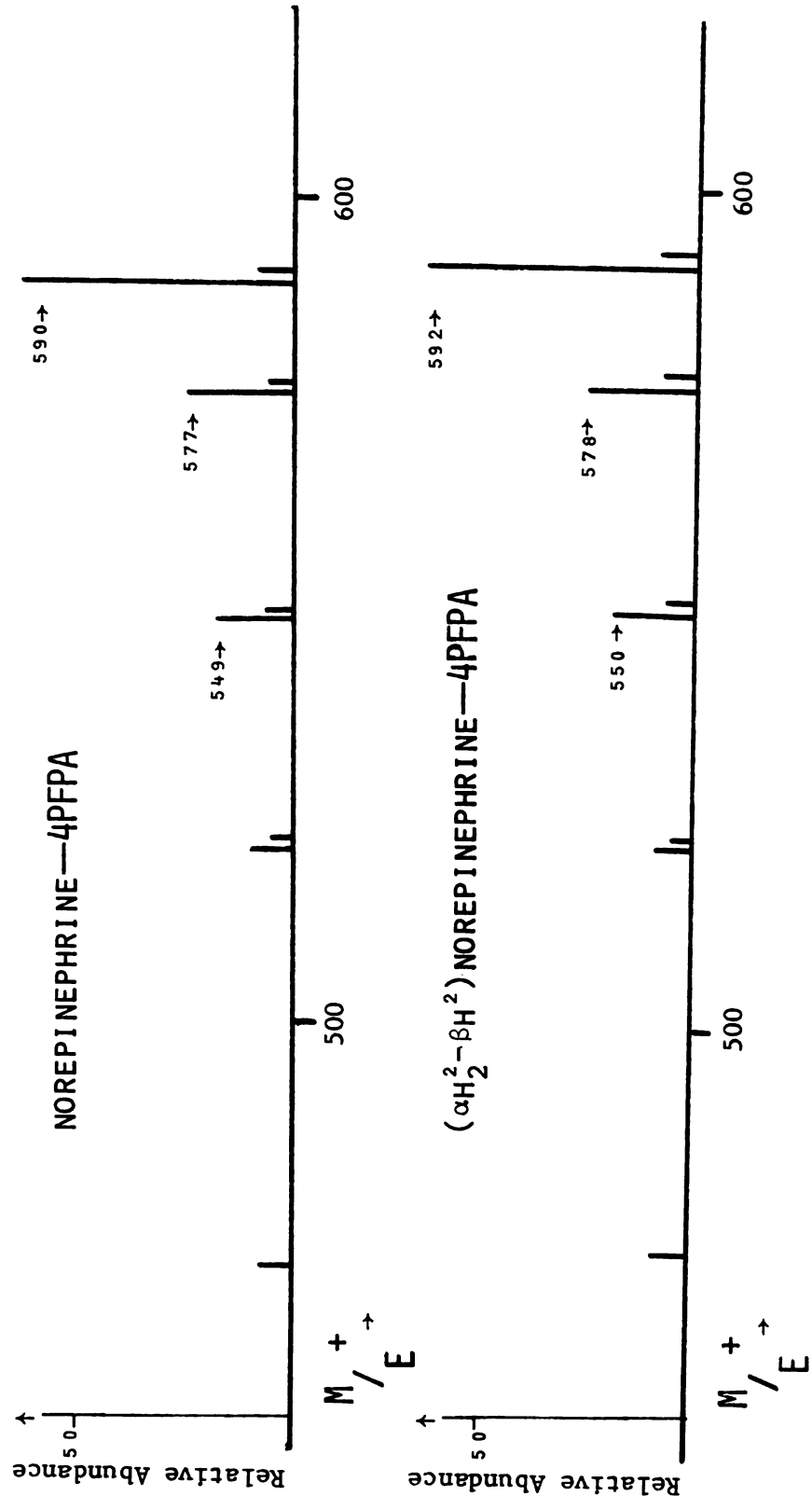
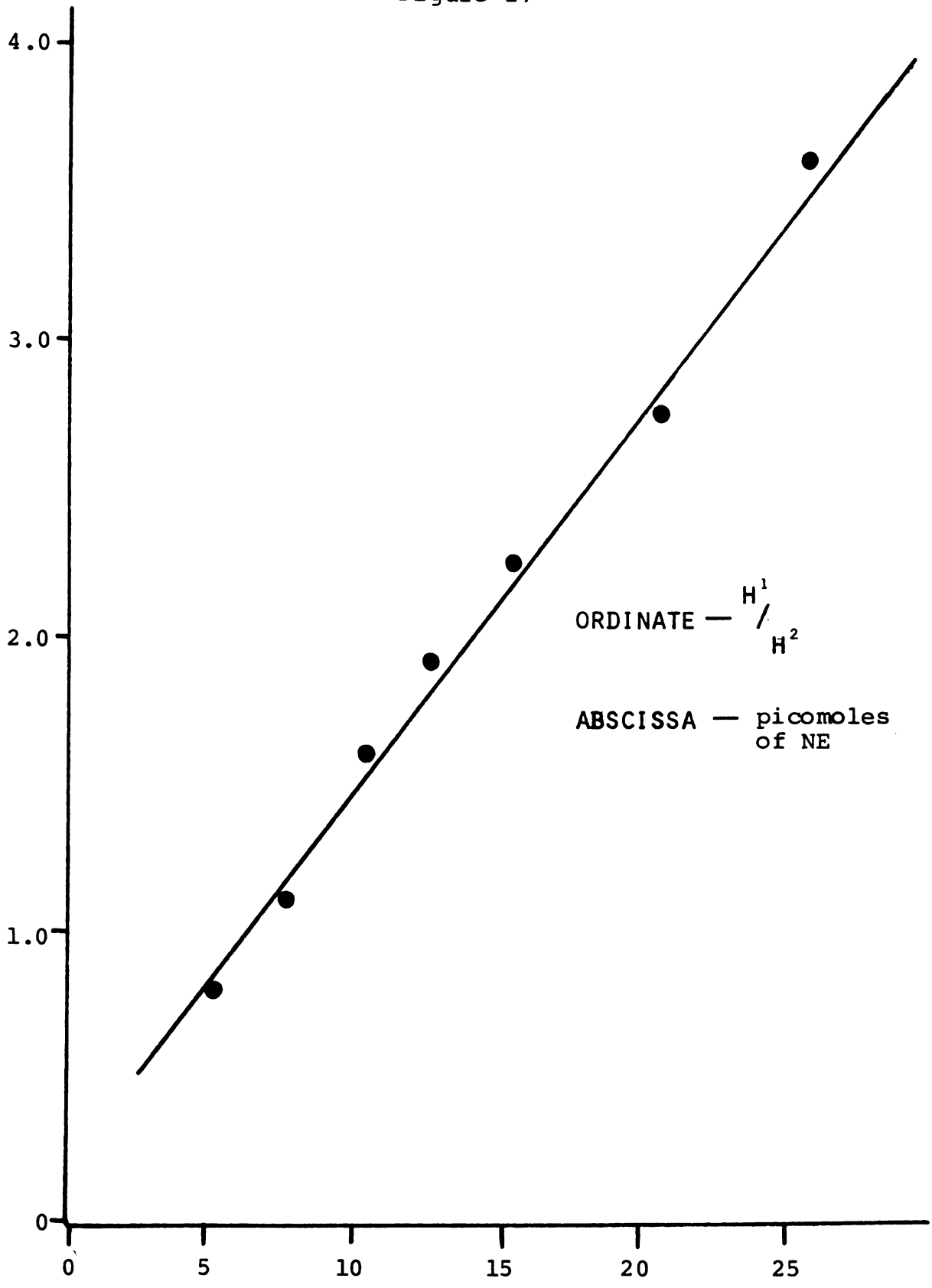

NOREPINEPHRINE—4PFPA
Relative Abundance
50
500
600
M/E
549→
577→
590→
(αH2-βH2)NOREPINEPHRINE—4PFPA
Relative Abundance
50
500
600
M/E
550→
578→
592→

Figure 27. Representative Standard Curve for norepinephrine quantitation by GC/MS mass fragmentography, 577/578 ion pair. The observed protium/deuterium (P/D) ion intensity ratio (ordinate) is plotted against the amount of NE injected into the GC/MS system (expressed as picomoles of NE). The volume of sample injected was allways 1.0 µl.

Figure 27


4.0
3.0
2.0
1.0
0
0
5
10
15
20
25
ORDINATE — H^1/H^2
ABSCISSA — picomoles of NE

RESULTS

The effect of caroxazone and phenelzine on the concentration of norepinephrine in rat hypothalamus is seen in Table 8 and Figure 28. As expected, phenelzine administration resulted in a dose-dependent increase in hypothalamic norepinephrine concentration. Caroxazone administration also increased hypothalamic norepinephrine concentration, although not to the extent as occured following phenelzine administration. A 10 mg/kg dose of phenelzine increased hypothalamic norepinephrine concentration to approximately 160% of control (15.96 nanomoles of norepinephrine per gram of hypothalamic tissue, wet weight), where as caroxazone increased hypothalamic norepinephrine to approximately 130% of control (13.00 nanomoles of norepinephrine per gram of hypothalamic tissue, wet weight).

These results agree with the earlier work of Suchowsky et al. (1969b). The magnitude of caroxazone-induced increases in hypothalamic norepinephrine concentration are approximately the same as caroxazone-induced increases in norepinephrine concentration in whole mouse brain reported by this investigator. The effect of caroxazone on the concentration of 5-HIAA in the caudate nucleus of the rat was also determined in a few subjects. 5-HIAA was quantitated using GC/MS mass fragmentography (Gelpi et al., 1974,

TABLE 8

Effect of Caroxazone and Phenelzine on the Concentration of Norepinephrine in Rat Hypothalamus

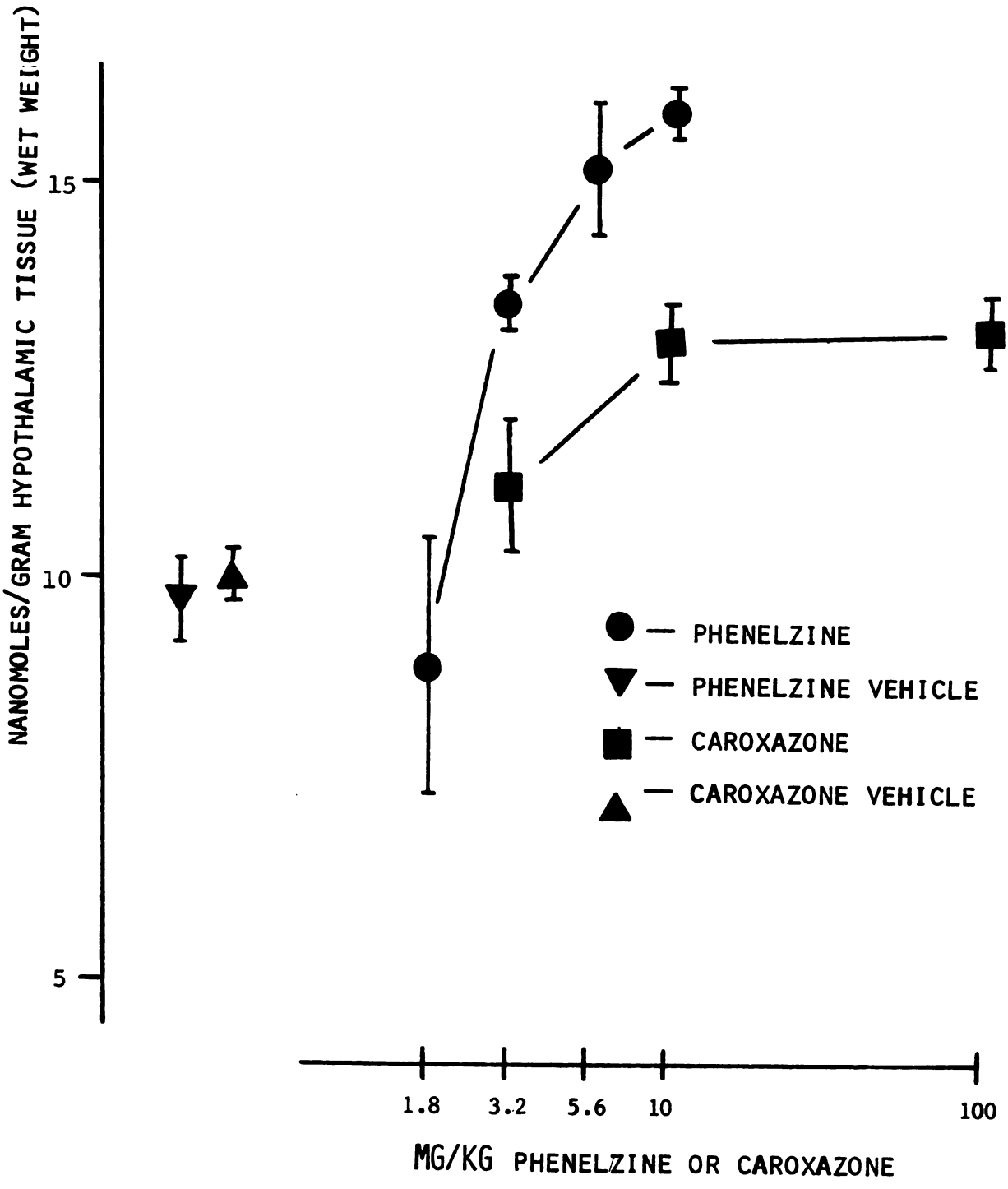
Treatment	Treatment Protocol t =0 hr, i.p. injection t = 2 hr, sacrifice Mean Concentration (S.E.M.)[1]	N
Phenelzine		
Vehicle	9.74(0.46)	3
1.8 mg/kg	8.94(1.66)[2]	3
3.2 mg/kg	13.45(0.70)	3
5.6 mg/kg	15.30(0.70)	3
10 mg/kg	15.96(0.19)	3
Caroxazone		
Vehicle	10.11(0.29)	4
3.2 mg/kg	11.17(0.86)	4
10 mg/kg	13.00(0.45)	4
100 mg/kg	13.29(0.50)	5

[1]Nanomoles of norepinephrine/gram of hypothalamic tissue (wet weight). Derivative monitored : norepinephrine—4—PFPA (577/578 and 590/592 ion pairs).

[2]Large S.E.M. is related to tissue weight variability (ie., two of the tissue weights were high) and underscores the importance of uniform disections.

Figure 28. Hypothalamic norepinephrine concentration in rat brain following various doses of phenelzine or caroxazone. Nanomoles of norepinephrine per g of tissue, wet weight, is plotted against dose, mg/kg, in log-spaced units. Phenelzine data is the mean concentration (±S.E.M.) of 3 values and n=4, except for 100 mg/kg dose where n=5, for caroxazone.

Figure 28


NANOMOLES/GRAM HYPOTHALAMIC TISSUE (WET WEIGHT)
15
10
5
PHENELZINE
PHENELZINE VEHICLE
CAROXAZONE
CAROXAZONE VEHICLE
1.8
3.2
5.6
10
100
MG/KG PHENELZINE OR CAROXAZONE

Watson and Wilk, 1974; Bertilsson et al. 1972). Control subjects had a mean concentration of 5-HIAA in the caudate nucleus of 4.07±0.17 nanomoles of 5-HIAA per gram of caudate nucleus, wet weight (±S.E.M., n = 4). Subjects treated with a 10 mg/kg i.p. dose of caroxazone and sacrificed 2 hours later had a mean concentration of 5-HIAA in the caudate nucleus of 4.16±0.31 nanomoles of 5-HIAA per gram of caudate nucleus, wet weight (±S.E.M., n = 4). This preliminary work agrees with that of Suchowsky et al. (1969b) and argues against caroxazone exerting central activity through monoamine oxidase inhibition, at least at this particular dose.

SUMMARY AND GENERAL CONCLUSIONS

Evidence is lacking that a significant amount of the total brain MAO activity is compromized by caroxazone at ED_{10} to ED_{90} anti-reserpine and anti-αMT doses (doses between 3.2 and 10 mg/kg). There is, however, convincing evidence that the spectrum of laboratory "antidepressant" activity of this agent is not dissimilar from that seen for classical MAOI, at least when drug activity is evaluated within a few hours after caroxazone administration or when maintenance doses are administered every few hours. The data presented in Sections I and II characterized caroxazone as exerting an overall cerebral pharmacodynamic profile that is different from both tertiary amine TCA and secondary amine TCA. However, thae most plausible interpretation of the data is that caroxazone is a reversible MAOI, that for the most part is not all that different pharmacodynamically from classical MAOI. The distinctions that were noted could be explained solely on the basis of drug-receptor interactions that are quantitatively and wualitatively different. That phenelzine and caroxazone are nearly equipotent in anti-reserpine and anti-αMT activity is clear. However, it is also obvious that caroxazone did not exhibit significant inhibition of "Type-A" MAO activity (chlorgyline sensitive, 5-HT and NE deaminating) based upon the results contained in Section III.

Fuentes and Neff (1975; see also Neff and Young, 1974) have shown that drugs that are relatively specific inhibitors of "Type-B" MAO activity (β-phenylethylamine and benzylamine deaminating; Johnson, 1968) such as deprenyl, do not protect against certain CNS depressant effects of reserpine in rats. However, it was shown that "Type-A" inhibitors and "mixed-type" inhibitors, such as phenelzine, exhibited complete protection against reserpine in these same tests. Dopamine is apparently deaminated by "Type-A" enzyme *in vivo* (Waldmeier *et al.*, 1976). Potentiation of the psychomotor stimulant effects of L-DOPA in combination with a peripheral decarboxylase inhibitor is also dependent upon expression of "Type-A" inhibitory activity (Maitre *et al.*, 1976). Thus, assuming these statements are in fact accurate, then two mutually exclusive statements of equality exist *if* the anti-reserpine effects of phenelzine and caroxazone are not different and *if* the anti-reserpine and DOPA-potentiation effects of phenelzine are by the same mechanism. The potency ratios seen in Table 9 summarize this dissociation of potency and effect.

It cannot be stated with any degree of certainty that the antidepressant efficacy of the MAOI in the treatment of psychiatric depression is a direct result of the irreversible inhibition of brain MAO. Brain MAO is probably heterogeneous with respect to substrate affinities, inhibitor affinities, distribution within the brain structure, axonal transport distances and turnover rates. Until more is known

TABLE 9

Potency ratio for caroxazone and phenelzine in specific test situations

	$\frac{\text{Caroxazone}}{\text{Phenelzine}}$ = dose ratio			
	Uncorrected dose		Corrected dose[1]	
	mg/kg	meq/kg[2]	mg/kg	meq/kg[2]
1. Equivalent doses for prevention of reserpine depression of rotarod performance: Lowest dose eliciting a significant preventative effect (at $p<.01$).	1.00	0.90	0.63	0.57
2. Equivalent doses for prevention of MT depression of rotarod performance: Lowest dose eliciting a significant preventative effect (at $p<.01$).	1.33	1.20	0.95	0.85
3. Equivalent effective doses: Lowest dose eliciting a significant increase in spontaneous motor activity when given in combination with L-DOPA and HMD (at $p<.01$).	17.50	15.70	15.60	14.00
4. Minimum lethal dose when given in combination with L-DOPA and HMD in female rats with 8 subjects/dose (at 24 hours).	31.10	27.90	---	---

[1]Caroxazone dose "corrected" for K_e=0.001925.

[2]Molecular weight of caroxazone = 206.

about the normal function of these enzyme systems, we are not likely to have a clear understanding of drugs that alter their activity. The results of this study indicate that caroxazone differs at least to some extent in interacting with brain monoamine mechanisms from the TCA and the MAOI in rats. Probably the most significant finding of these studies was the relatively low toxicity of caroxazone in the L-DOPA potentiation experiments since this has direct clinical relevance.

As a last thought, it should be kept in mind that it is always risky to extrapolate from animals to man in drug research. Because of the extreme complexity of the mammalian CNS and the uniqueness of the human experience, such extrapolations are often of questionable value in the discipline of psychopharmacology. Therefore, clinical comparisons of caroxazone with the traditional MAOI will be required before very much more can be said about the psychodynamic effects of this new drug.

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