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ABSTRACT

THE INTERACTION BETWEEN MONOAMINE OXIDASE INHIBITORS AND MEPERIDINE

Edith Zorychta

The toxic reaction which can occur when patients taking monoamine oxidase (MAO) inhibitors are given meperidine has never been satisfactorily explained. It is well known that MAO inhibitors elevate brain amines, and compounds such as morphine and meperidine have also been shown to affect these agents. It therefore appeared reasonable that the interaction between phenelzine and meperidine might be linked to their combined effect on one or more of these amines. Male Sprague-Dawley rats were treated with phenelzine or meperidine, killed by immersion in liquid nitrogen, and the frozen brains were removed and analysed for 5-hydroxytryptamine, noradrenaline and dopamine. Phenelzine (10 to 50 mg/kg) elevated all three amines, with a maximum change at six hours following administration. The effects of meperidine (15 to 50 mg/kg) were less striking. It had no significant effect on brain levels of noradrenaline, and tended to slightly increase levels of dopamine and lower those of 5-hydroxytryptamine. When a low dose of meperidine (15 mg/kg) was given to rats pretreated with phenelzine (25 mg/kg), brain levels of all three amines were significantly different from those in rats receiving phenelzine alone. Both catecholamines were further elevated, with the most marked change in dopamine, while 5-hydroxytryptamine levels decreased. The results of these experiments support the involvement of all three brain amines in the phenelzine-meperidine interaction.

Short Title:

THE INTERACTION BETWEEN MAO INHIBITORS AND MEPERIDINE

THE INTERACTION BETWEEN MONOAMINE OXIDASE INHIBITORS AND MEPERIDINE

by

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PREFACE

The work reported in this thesis is part of an investigation into the cause of a potentially lethal drug interaction which can occur when a patient receiving one of the monoamine oxidase (MAO) inhibitors is subsequently given a narcotic analgesic. Two mechanisms have been previously proposed to explain this interaction. It has been suggested that interference by the MAO inhibitor with metabolism of the analgesic might be the cause. The effect of MAO inhibitors on the metabolism of the narcotic analgesic meperidine (Demerol) has been studied extensively in our laboratory (Eade and Renton 1970a, b). From these experiments and others it seemed unlikely that this mechanism could account for the observed toxicity. Another hypothesis suggested that the combination of these two centrally active drugs might cause an alteration in brain amine levels leading to toxic symptoms. The project reported here was designed to study this possibility.

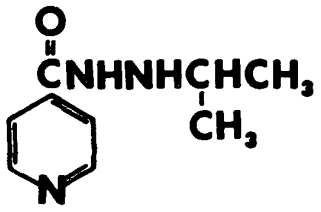
This thesis begins with a review of the development and use of the MAO inhibitors and their interaction with narcotic analgesics. Topics discussed include present knowledge concerning the major brain amines, the role of MAO, and the effect of MAO inhibitors and narcotic analgesics on brain amine levels. As the literature is extensive for all of these subjects the review is not intended to be complete, but merely to provide information most relevant to the project.

I. INTRODUCTION

A. DEVELOPMENT OF MAO INHIBITOR ANTIDEPRESSANTS

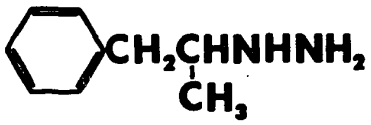
Interest in the MAO inhibitors began with iproniazid (Marsilid), an antitubercular drug synthesized in 1951 by Fox of Hoffmann-La Roche Laboratories. During clinical trials, iproniazid was reported to produce marked side effects of central nervous system stimulation (Selikoff et al., 1952). As these were considered undesirable, the drug was largely abandoned in favor of the parent compound isoniazid (Niconyl), except for limited trials on tuberculous patients who suffered from depression as well. Experimental studies of the drug continued, however, and Zeller (1952) demonstrated that iproniazid, in contrast to isoniazid, was a potent inhibitor of MAO. This initiated widespread interest in the drug, based on the hypothesis that its central stimulant properties might be related to its enzyme-inhibitory action. Brodie et al. (1956) showed that in animals iproniazid elevated brain monoamine levels while causing excitement, and reversed the action of reserpine, a compound which normally lowered brain amine levels and caused sedation. These results, along with heightened interest in the role of monoamines in the central nervous system and their possible relationship to mental illness, prompted three independent groups of investigators to study the effect of iproniazid on mental depression. In 1957, at a meeting of the American Psychiatric Association, all three groups reported iproniazid to have definite therapeutic value (Loomer et al.,

1957; Crane, 1957; Scherbel et al., 1957). Labelled a "psychic energizer" by Kline, it was soon widely used in the treatment of depression.



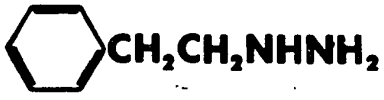
IPRONIAZID

The introduction of iproniazid stimulated a vast research effort to find more effective MAO inhibitors, and other hydrazines were quickly investigated. One series of compounds resulted from a study by Biel et al. (1959) of the structure-activity relationships of the phenylalkyl hydrazines, in an attempt to find sympathomimetics which would not be substrates for MAO and would thus be more potent and longer lasting than those available at the time. These derivatives were found to be strong MAO inhibitors (Horita, 1958; Biel et al., 1958, 1959), and the compound JB-516 or pheniprazine (Catron) was extensively investigated. It was much more potent than iproniazid, both in vivo and in vitro and was shown by Spector (1958) and Biel (1958) to produce a rapid rise in brain levels of noradrenaline and 5-hydroxytryptamine in the rabbit.

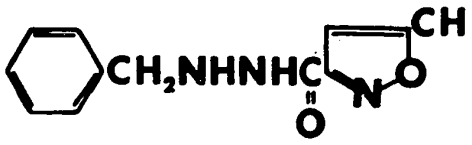


PHENIPRAZINE

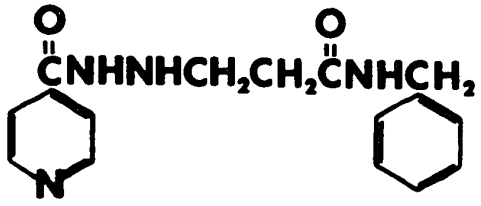
Subsequently, numerous analogues of iproniazid were synthesized, but unfortunately the serious side effects of the early hydrazines and hydrazides, associated with hepatotoxicity, impairment of red-green color vision, and neurologic damage, dampened enthusiasm for further development. The only compounds of this series still in clinical use are phenelzine (Nardil), isocarboxazid (Marplan) and nialamide (Niamid).



PHENELZINE



ISOCARBOXAZID

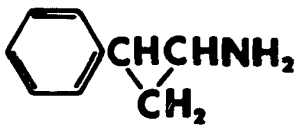


NIALAMIDE

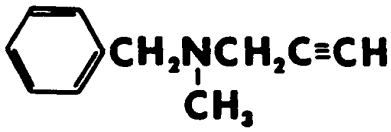
Attention was therefore focused on finding nonhydrazine MAO inhibitors. The first of these shown to have potent *in vivo* action were the harmala alkaloids harmine, harmaline, and related carboline derivatives (Udenfriend *et al.*, 1958). Long

before their MAO inhibitory action was recognized they were observed to have central nervous system excitatory and hypotensive properties. However, due to poor intestinal absorption, short duration of action, and marked toxic side effects they were not useful clinically.

Tedeschi et al. (1959) and Maass and Nimmo (1959) initially reported the potent in vivo inhibitory properties of tranylcypromine (Parnate), which had been synthesized in 1948 by Burger and Yost. The first non-hydrazine MAO inhibitor to be marketed, it gained considerable acceptance clinically, as it was as effective as the hydrazines but had a lower incidence of toxic side effects. Pargyline (Eutonyl), another non-hydrazine compound described by Swett et al. (1960), is a strong MAO inhibitor (Taylor et al., 1960) but is less useful than tranylcypromine in the treatment of depression.



TRANYLCYPROMINE



PARGYLINE

For several years the MAO inhibitors were the only effective antidepressants available. Various central stimulants such as amphetamine, methylphenidate, and pipradol had been tried but were of little benefit except in very mild cases. The

introduction of another important group of antidepressant agents began with clinical trials of imipramine (Tofranil), one of a group of aminoalkyl derivatives of iminodibenzyl which had been synthesized as antihistamines, sedatives, and antiparkinsonism agents by Hafliger in 1951. A few of these showed hypnotic properties and when they were tested in psychotic patients Kuhn (1957) fortunately noted that imipramine was of considerable benefit in certain depressed states. Following extensive study, imipramine was introduced as an antidepressant in Europe in 1958 and in North America in 1959. The resultant search for chemically related compounds led to the marketing of amitriptyline (Elavil), desipramine (Norpramin), and nortriptyline (Aventyl). Imipramine and amitriptyline are now the most widely used antidepressant agents. They are clinically at least as effective as the MAO inhibitors with considerably lower potential toxicity (Cole, 1964).

B. INTERACTIONS BETWEEN MAO INHIBITORS AND OTHER SUBSTANCES

MAO inhibitors can provoke a serious, sometimes fatal reaction when other drugs or foods are subsequently ingested. Agents contraindicated during MAO-inhibitor therapy include sympathomimetics (Everett *et al.*, 1963), amine-containing foods such as cheese, wine, and certain vegetables (Horwitz *et al.*, 1964; Hodge *et al.*, 1964), imipramine (Himwich, 1962; Brachfeld *et al.*, 1964), meperidine (Shee, 1960), and barbiturates (Domino *et al.*, 1962). The reasons for many of these

interactions have been explained through a knowledge of the pharmacological properties of both compounds involved. For example, an agent such as amphetamine which releases noradrenaline from nerve terminals may provoke a reaction after MAO inhibitors, as there are larger amine stores available to be released. With this knowledge an α-adrenergic blocking agent is the logical choice for the treatment of this interaction (Sjoqvist, 1965). The mechanisms involved in other interactions, including that observed with meperidine have not yet been satisfactorily explained.

The danger of a meperidine reaction was not generally recognized until 1962, although the first case was reported when iproniazid was still under trial as an antitubercular agent. Mitchell (1955) described nausea, cyanosis, sweating, muscle twitching and tachycardia twenty minutes after the patient received 100 mg of meperidine. Following the introduction of iproniazid as an antidepressant, Papp and Benaim (1958) noted a similar, more serious reaction which occurred twice in the same patient. In 1960 Shee warned against the combination of iproniazid and meperidine, having observed two severe interactions where an initial hyperactive stage involving restlessness, flushing, profuse sweating, dilatation of the pupils, tachycardia and convulsions, was followed by coma. His article prompted Palmer (1960) to report a previous unexplained death which occurred when meperidine was given to a patient receiving

phenelzine. Pells-Cocks and Passmore-Rowe (1962) also noted a severe phenelzine-meperidine interaction and recognized the connection between MAO inhibitors as a group and meperidine toxicity, pointing out that the latter compound should not be administered after any of the six such antidepressants then in general use. Although this hazard was then realized by the medical profession, numerous clinical reports of adverse interactions still appeared, mainly because the drugs were usually administered by different physicians, the MAO inhibitor by a psychiatrist, and the analgesic by a surgeon, obstetrician, or anaesthetist.

There is no satisfactory explanation for the combined toxicity of these two drugs. The reaction has been attributed to a decreased metabolism of meperidine caused by inhibition of liver microsomal enzymes by the antidepressants. This hypothesis has been extensively studied, and while it may be involved, it has been discredited as a major factor, as explained in Part IV, Section A.

The therapeutic efficacy of MAO inhibitors in depression is generally attributed to their action on brain amines. Analgesics such as morphine and meperidine are also known to affect brain amines and attempts have been made to link their analgesic and addictive properties to changes in brain amine levels, although their precise mode of action is still undefined. It seemed reasonable, then, that the interaction between MAO

inhibitors and meperidine might be due to their combined effect on brain amines, and it was decided to examine the effects of phenelzine and meperidine, alone and in combination, on brain amine levels, in an attempt to explain their toxic interaction.

C. BRAIN AMINES

The monoamines noradrenaline, dopamine and 5-hydroxytryptamine have been extensively studied in the mammalian brain, and an immense amount of information is available regarding their cellular and subcellular distributions, enzymatic formation and destruction, and cellular uptake and release, under normal conditions, and following a wide variety of drugs. Some of the evidence supporting each amine as a possible central transmitter will be described here. Relevant pharmacological data will be considered in a following section.

1. Noradrenaline

Noradrenaline was demonstrated as a natural constituent of brain tissue by von Euler in 1946. Vogt, in 1954, mapped out the regional distribution of this amine in the dog brain through biochemical analysis, generating an intense interest in its possible function. Noradrenaline was found in highest concentrations in the brain stem, mainly in the hypothalamus, with somewhat lower levels in the midbrain and medulla. The enzymes responsible for synthesis and metabolism of this amine were distributed in a similar manner.

With the introduction of fluorescence microscopy, a technique permitting visual study of amines within the cell (Falck, 1962; Falck et al., 1962), much information on the storage and release of biogenic amines has been accumulated.

Noradrenaline is present exclusively within neurons. It is found in low concentrations in the perikaryon, is absent in the nucleus, and is very highly concentrated in the rounded enlargements, or terminals of the axon. Cell bodies of noradrenaline-containing neurons are found mainly in the mid-brain, and their terminals are scattered throughout the brain, with a high concentration in the hypothalamus (Carlsson et al., 1962; Dahlstrom and Fuxe, 1964).

Information regarding the actual storage sites for central amines began with investigations of acetylcholine by Hebb and Whittaker (1958) who found that by a combination of differential and density-gradient centrifugation they could isolate a fraction from brain tissue which consisted of pinched-off nerve endings, or synaptosomes (Gray and Whittaker, 1960a, Whittaker et al., 1964). Crusciel, in 1960, found in the dog hypothalamus that noradrenaline was also localized in this tissue fraction, a fact which has since been confirmed in other animals by a number of investigators (Potter and Axelrod, 1963; Levi and Maynert, 1964). The synaptosome fraction has been shown microscopically to contain most of the green material seen in fluorescence microscopy, which is noradrenaline (Whittaker, 1966).

Electron microscopy has indicated the actual amine storage sites. When brain tissue is fixed and stained with heavy metals, some nerve endings are shown to contain granular vesicles characterised by a core of electron-dense material; these granular vesicles are considered to be storage sites for noradrenaline (De Robertis, 1966). Supporting evidence for this proposal is strong. Noradrenaline is a reducing agent and its presence in high concentrations would be expected to induce an intense local deposition of heavy metals, as is known to occur in the chromaffin granules of the adrenal medulla. The vesicles are depleted of noradrenaline by reserpine, and this effect is antagonized by MAO inhibitors. Variations in the number of granular vesicles correlate with variations in noradrenaline content in different parts of the brain (Ishii et al., 1965). When the synaptosome fraction is disrupted by osmotic shock, a subfraction consisting mainly of synaptic vesicles can be isolated and this vesicular fraction has the highest relative concentration of noradrenaline in the brain (Maynert et al., 1964; De Robertis, 1966).

The availability of radiolabelled amines has allowed detailed study of their synthesis, uptake, release, and metabolism in brain. Noradrenaline injected systemically will not cross the blood-brain barrier, but 3-4-dihydroxyphenylalanine (DOPA), one of its precursors will. By injecting labelled DOPA, and measuring the brain levels of labelled noradrenaline, the rate of synthesis of the amine can be determined. Radioactive

noradrenaline injected intraventricularly is taken up by neurons and mixes with the endogenous amine. It has been proven to be a valid tracer for studying re-uptake, release, and metabolism (Glowinski, 1966). Normally, 50 percent of tritiated noradrenaline is taken up by rat brain and the major metabolite first released is O-methylated, reinforcing the belief that endogenously released noradrenaline is inactivated mainly by re-uptake into nerve endings, and also by extra-neuronal catechol-ortho-methyl transferase (COMT). During a later phase, deaminated O-methylated metabolites predominate. MAO probably inactivates noradrenaline which is released from the granules intraneuronally, without playing a role in terminating physiological activity at the receptor.

An inhibitory pathway in the olfactory bulb of the rabbit is one central synaptic area where the evidence for adrenergic transmission is convincing. Electrical stimulation of the lateral olfactory tract (LOT) produced a prolonged inhibition of spontaneous firing of ipsilateral mitral cells, accompanied by hyperpolarization of the cell membrane (Yamamoto et al., 1963; Salmoiraghi, 1964). Administration of noradrenaline produced the LOT inhibitory response, and this response was blocked by dibenamine and phentolamine. Pretreatment with reserpine or α-methylmetatyrosine decreased the LOT inhibitory response to electrical stimulation while having no effect on the response to noradrenaline (Salmoiraghi, 1964). This pathway

in the rabbit olfactory bulb is thus considered to utilize noradrenaline as a neurotransmitter (Salmoiraghi, 1964, 1966).

A further body of evidence supporting noradrenaline as a chemical transmitter in brain has been gathered. A wide variety of stimuli such as environmental temperature changes, stress, ECT, and electrical stimulation of certain parts of the brain are known to alter central amine levels. Because much of this evidence does not offer proof for the role of noradrenaline, and the techniques and interpretation are controversial, it will not be discussed here. While the evidence for noradrenaline as a central transmitter is convincing, proof would require intracellular recording from one neuron during administration of the amine and related drugs extraneuronally, and a correlation of these responses with those evoked by electrical stimulation of the synaptic input to that neuron. Due to technical difficulties, this evidence is not as yet available for any substance present in the central nervous system.

2. Dopamine

Dopamine was found to be a normal constituent of the mammalian brain in 1957 (Montagu, 1957; Weil-Malherbe and Bone, 1957). The distribution of this amine in the central nervous system differs from that of noradrenaline. Carlsson (1959) found high levels in the corpus striatum, an area containing little noradrenaline, suggesting that dopamine has a function

of its own, apart from being a precursor in the synthesis of noradrenaline. High dopamine concentrations occur in the basal ganglia, with lesser amounts in the midbrain, hypothalamus, and pons.

Dopamine-containing neurons have characteristics similar to those containing noradrenaline, with a low concentration of amines in the cell body and axon, none in the nucleus, and a high level in the terminal varicosities. Dopamine neuron systems all give rise to very fine terminals which are densely packed; amine concentrations here are in the order of 10,000 µg/g (Carlsson et al., 1964). They make axodendritic but not axosomatic contacts. They can be distinguished from those containing noradrenaline in two ways. 1) They recover faster than noradrenaline terminals after the administration of α-methyl-metatyrosine. 2) After α-methyldopa and reserpine, dopamine terminals lose their fluorescence; however, noradrenaline terminals still fluoresce. Noradrenaline, dopamine, and α-methyldopamine are depleted by reserpine, whereas α-methyl-noradrenaline is not, and it is this compound that fluoresces (Carlsson et al., 1965).

Non-terminal axons and cell bodies contain about 100 µg/g of dopamine, and the axons are seen to contain ovoid fluorescent enlargements. These vesicles may be made in the cell body and carried toward the terminals, as cutting the axon results in a great increase of amines on the central side of the lesion.

The highest specific concentration of dopamine is found in the synaptosome fraction; however, a greater portion of the total dopamine is found in other layers, when compared to noradrenaline or 5-hydroxytryptamine. This may be due to a different mechanical or geometrical property of the dopamine granules.

Human pathology provided the first indications on the role of central dopamine. Ehringer and Hornykiewicz (1960) found a large decrease in dopamine, sometimes as low as 10% of normal, in the caudate nucleus and putamen of parkinsonian patients. The main metabolite of dopamine, homovanillic acid (HVA) was also lowered in this condition (Bernheimer and Hornykiewicz, 1965). The cells giving rise to dopamine terminals in the corpus striatum may be in the substantia nigra, and it is here that the crucial lesion in Parkinson's disease may occur. Fluorescence microscopy indicates a possible nigro-striatal pathway, and as shown both microscopically (Anden et al., 1964) and chemically (Poirier and Sourkes, 1965), lesions made in the substantia nigra of animals cause a substantial decrease of dopamine in the corpus striatum.

Attempts to study the role of dopamine more directly have shown that stimulation of the substantia nigra can cause release of this amine from a push-pull cannula situated in the putamen (McLennan, 1965). This suggests a role for dopamine as a transmitter here, but the insertion of the cannula could itself

cause leakage of substances present in tissue. Experiments by Portig and Vogt (1969), using perfusion of the lateral ventricle to avoid tissue damage, showed a release of dopamine and a much larger release of its main metabolite, HVA, from the caudate nucleus after electrical stimulation of the substantia nigra. The resting levels of HVA in the perfusate fell as anesthesia deepened, suggesting that this metabolite is formed as a result of activity in dopamine neurons.

3. 5-Hydroxytryptamine

5-Hydroxytryptamine was found in brain by Twarog and Page in 1953. Highest levels occur in the hypothalamus, where 5-hydroxytryptophan decarboxylase and MAO are also in abundance. Substantial amounts also occur in the brain stem, amygdala, caudate nucleus and mesencephalon, with little in the cortex (Amin et al., 1954). Like noradrenaline, it does not occur in medullated fibers. In contrast to noradrenaline terminals which make intimate contact with other catecholamine cells and 5-hydroxytryptamine cells, these terminals have only been observed to contact neurons which do not contain monoamines.

In contrast to the green fluorescent derivatives of the catecholamines, the 5-hydroxytryptamine derivative has a yellow fluorescence. MAO inhibitors increase the intensity of 5-hydroxytryptamine fluorescence much more than for the catecholamines (Dahlstrom and Fuxe, 1964).

Like the other two amines, bound 5-hydroxytryptamine is localized in the synaptosomal fraction of brain (Whittaker, 1958). It was proposed as a transmitter substance by Brodie and Shore in 1957, and several hypotheses suggest that central activity may be controlled by a balance between 5-hydroxytryptamine and acetylcholine, or between 5-hydroxytryptamine and noradrenaline, with one substance increasing and the other decreasing transmission. These ideas are still highly speculative.

It has been suggested that 5-hydroxytryptamine in excess is a stimulant, euphoriant, antidepressant, and possibly a psychotomimetic, while deficiency of this amine results in sedation, dysphoria and depression. These suggestions are based mainly on behavioural studies. Alterations in indolealkylamine metabolism have been reported in some psychiatric diseases and syndromes, and a large body of evidence indicates that changes in brain levels or actions of 5-hydroxytryptamine can produce behavioural changes in man and animals. While these correlations strongly implicate this amine as a central transmitter, it is still impossible to distinguish accurately between cause and effect.

D. THE ROLE OF MAO

In 1928, Hare demonstrated the existence of an enzyme which catalyzed the conversion of tyramine to p-hydroxybenzaldehyde, peroxide, and ammonia. Known as monoamine

oxidase, this enzyme is now recognized to be responsible for the oxidative deamination of a number of biogenic amines to their pharmacologically inactive aldehyde derivatives. Substrates include adrenaline, noradrenaline, dopamine, tyramine, tryptamine, and 5-hydroxytryptamine (Gorkin, 1966). The reaction is as follows:

$$RCH_2NHR^1 + O_2 + H_2O \xrightarrow{MAO} RCHO + R^1NH_2 + H_2O_2$$

$R^1 = H_2$ or a methyl residue.

MAO is an intracellular enzyme, found almost exclusively in the mitochondrial fraction (Oswald and Strittmatter, 1963; Baudhuin et al., 1964) and is probably bound to the outer membrane of the mitochondrion. The distribution of MAO varies. It has been demonstrated in most tissues, including kidney, intestine, brain and heart, with highest concentrations found in the liver and lowest in blood plasma and striated muscle (Pletscher et al., 1966). The enzyme is relatively stable, but so far little is known regarding the configuration of active sites. Sulfhydryl groups, flavin or metal ions may be involved (Lagnado and Sourkes, 1956; Wiseman and Sourkes, 1963), but there is no confirming evidence for any of these, or for requirements regarding coenzymes. Separation from other amine oxidases is based on substrate and inhibitor specificity, and the lack of susceptibility of MAO to inhibition by semicarbazide. Quantitative and qualitative differences exist in MAO from different organs and species, and it probably is not

one, but a group of closely related enzymes (Pletscher et al., 1966).

Monoamine oxidase and COMT are the major enzymes responsible for the metabolic degradation of catecholamines. The availability of radiolabelled amines has been a useful tool in determining their methods of inactivation. When tritiated noradrenaline is administered intravenously, part of it is excreted unchanged, part is taken up by sympathetic nerve endings, and the rest is metabolized and excreted. The major metabolites first appearing in the urine are O-methylated derivatives, while some time later deaminated catechols predominate. These results indicate that extracellular noradrenaline is destroyed by COMT while bound amine is first metabolized by MAO. (Kopin and Axelrod, 1963). Inhibition of MAO does not enhance the effect of sympathetic nerve stimulation, supporting the belief that it is not of prime importance in terminating the action of released noradrenaline, but functions to maintain the steady-state level of monoamine stores in neurons (Brodie et al., 1959; Spector et al., 1960).

In contrast to the catecholamines, MAO is the principal mechanism for 5-hydroxytryptamine degradation, although the latter can also be conjugated as a glucuronide or acetylated (Page, 1958). Some of the important pathways for inactivation of these substances are illustrated in Figures 1 and 2.

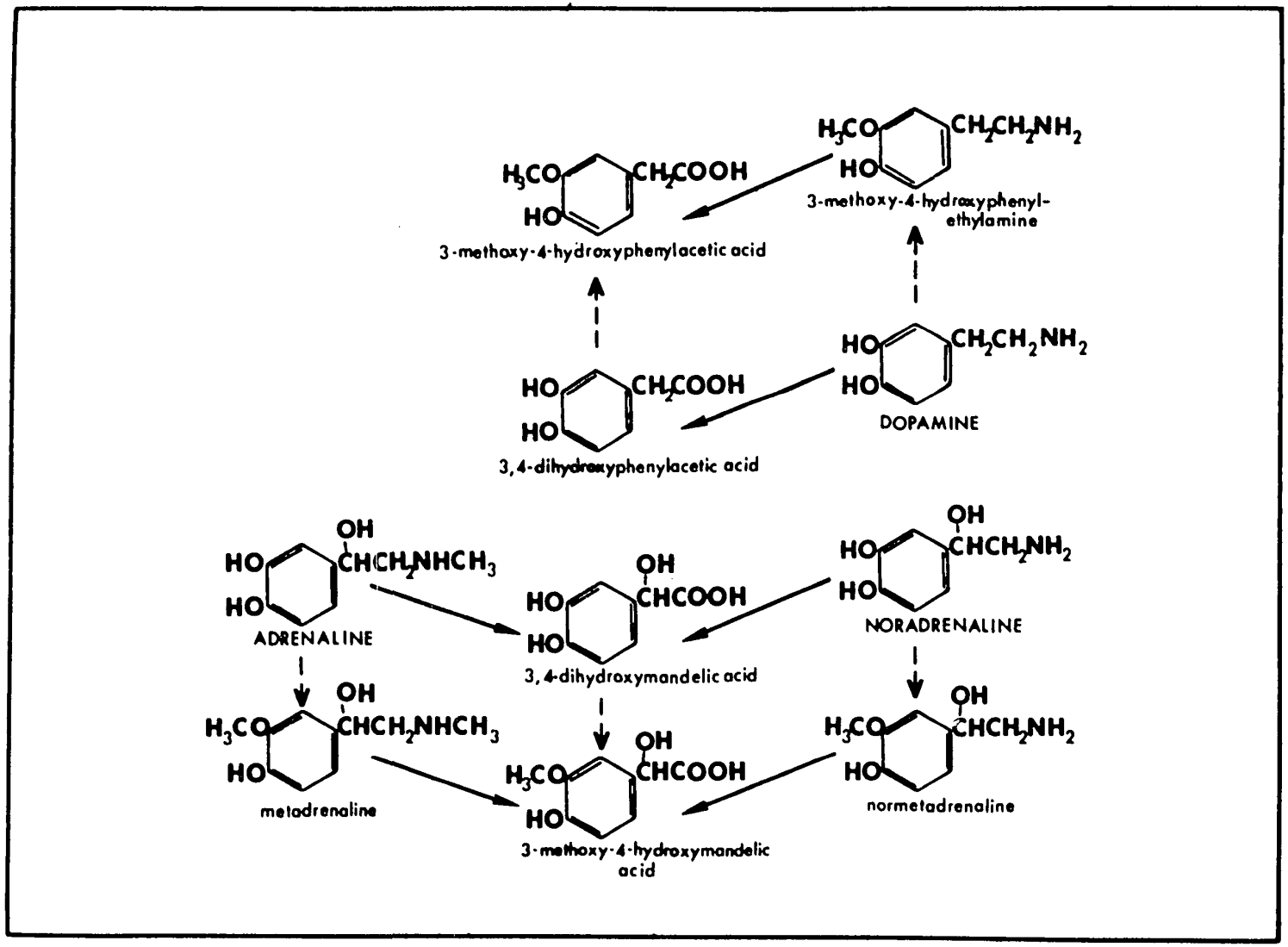


Figure 1: Metabolism of catecholamines.
——→ MAO
– – → COMT

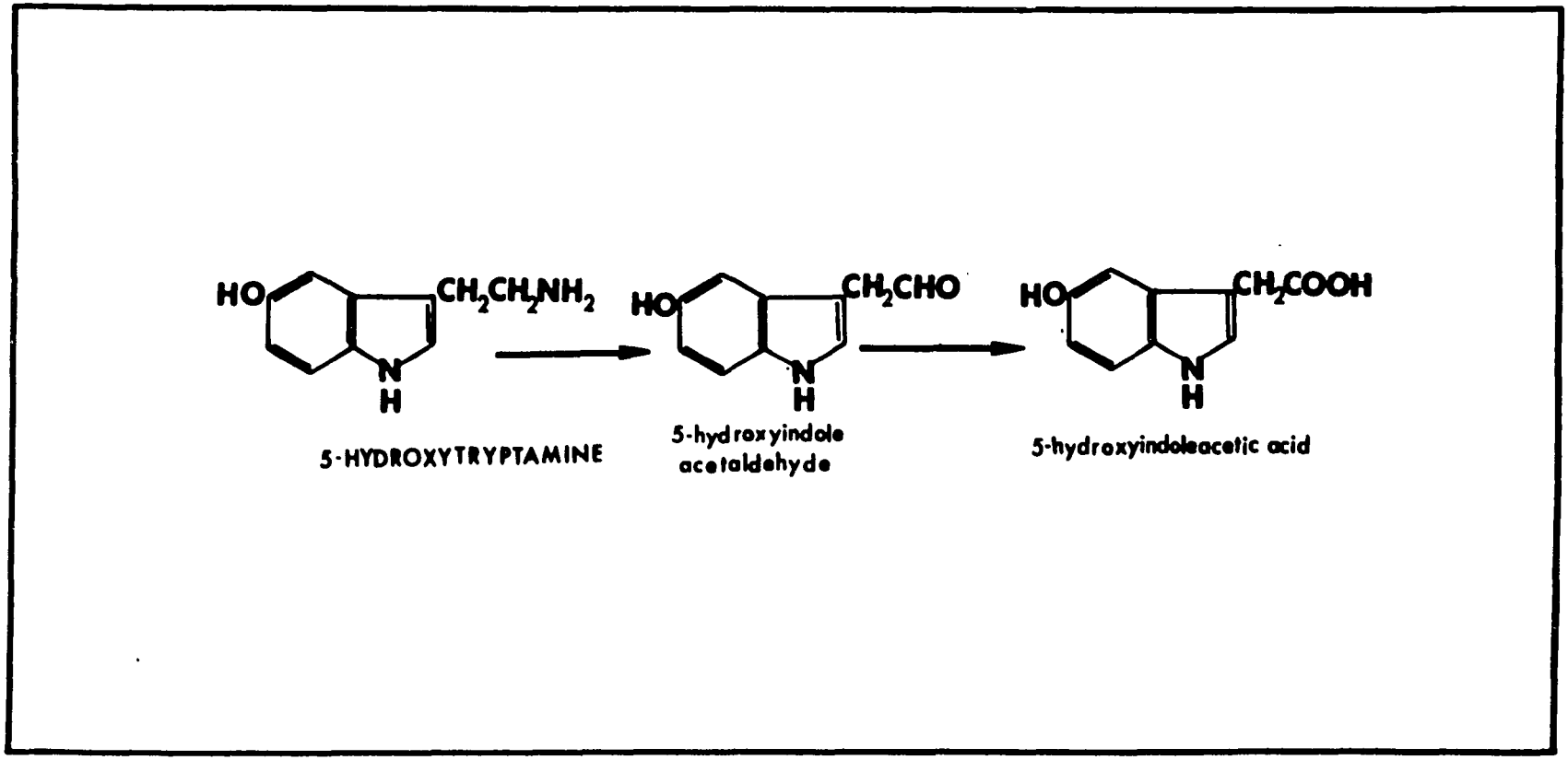


Figure 2: Metabolism of 5-hydroxytryptamine.

E. EFFECTS OF MAO INHIBITORS ON BRAIN AMINES

The MAO inhibitors in clinical use have an irreversible action on MAO which persists until the enzyme has been resynthesized. The effects of repeated doses are therefore cumulative and remain after the disappearance of the parent compound and its metabolites (Pletscher et al., 1960). The effects which have been directly attributed to the inhibition of MAO are: 1) an increase of endogenous monoamines in tissue; 2) a decrease in formation and urinary excretion of deaminated amine metabolites and an increase in excretion of monoamine precursors; 3) an increased response to some exogenous monoamines and their precursors (Schildkraut et al., 1963; Horwitz et al., 1960) and; 4) a decrease or reversal of the effect of monoamine liberators such as reserpine and tetrabenazine (Brodie et al., 1956). As this is a structurally diversified group of drugs, other effects exist which depend on the individual compound and are unrelated to MAO inhibition. Enzymes such as diamine oxidase, amino acid-decarboxylase, choline oxidase, and others may also be inhibited by the hydrazines. Liver microsomal enzymes are inhibited to different extents by the various compounds (Gey et al., 1963; Pletscher et al., 1966).

Many studies have been done on the effects of MAO inhibitors on brain amines and these drugs have been useful tools in elucidating the role of MAO and its amine substrates in the functions of the brain. Their acceptance for the treatment of

endogenous depression led to attempts to correlate their clinical effect with a measurable pharmacological response. The evidence gained from these efforts supported the theory that noradrenaline, dopamine, and 5-hydroxytryptamine are chemical transmitters in the central nervous system and led to many postulates regarding their roles in the control of behavioural states.

Investigation of the effects of MAO inhibitors on brain amines began in 1956, when Brodie et al. found that iproniazid elevated levels of 5-hydroxytryptamine in the rabbit brain to twice that of normal and increased noradrenaline levels also, but to a lesser degree. Central excitation was notable in the animals. This increase in brain monoamines after MAO inhibitors has been subsequently confirmed both histochemically (Dahlstrom and Fuxe, 1964; Norberg, 1965) and by electron microscopy (Pellegrino de Iraldi and De Robertis, 1964). Reserpine, a powerful depressant, was known to lower brain amines (Pletscher et al., 1956; Holzbauer and Vogt, 1956) and it was shown that MAO inhibitors, if given before reserpine would reverse its action to intense motor stimulation (Chessin et al., 1956). The amines released by reserpine could no longer be inactivated intraneuronally and would reach postsynaptic receptors in an active form (Pletscher, 1957). A hypothesis was then developed (Brodie and Shore, 1957; Brodie et al., 1958) implicating noradrenaline, dopamine and 5-hydroxytryptamine as central

neurotransmitters and suggesting that chemical alterations of these substances by drugs could lead to logical therapy of several forms of mental illnesses.

It was believed that the MAO inhibitors produced their antidepressant effect through their action on amines in the brain and not by some other mechanism. Further studies have supported this idea. When MAO inhibitors were administered after reserpine, the existing depression could not be reversed until the levels of brain amines were raised to nearly normal (Spector, 1963). Closely related structural congeners such as isoniazid, which did not inhibit MAO but were known to act on other enzymes such as diamine oxidase had no antidepressant effect. A critical level of amines seemed necessary for therapeutic effect, as a variable latent period was observed clinically between administration and onset of response; this was probably due to a slow buildup of amines, as seen in animals when lower doses were given repeatedly. Endogenous catecholamine levels can be decreased by α-methyltyrosine, which blocks the conversion of tyrosine to DOPA, the rate limiting step in catecholamine synthesis (Spector et al., 1965). When this compound was administered to rats, tranylcypromine given 150 minutes later did not produce central excitation, supporting the belief that a certain level of amine is necessary for this effect (Schildkraut, 1970).

Although it is generally accepted that MAO inhibitors

act through their effect on brain amines, the exact sequence of events is not certain. The drugs are thought to increase amine levels in a deep storage pool to the point where spillover into a mobile pool occurs, thus causing more amine to be present at postsynaptic receptors producing excitation (Brodie, 1963). Other factors may contribute as well. Some MAO inhibitors have been shown to affect monoamine release in brain to some extent, or to block uptake of released amine into storage granules, which would yield increased concentrations at synapses. Tranylcypromine was shown to produce in mouse brain an immediate decrease in noradrenaline levels, followed by a substantial increase (Carlsson et al., 1960), suggesting an initial releasing phase. The effects of tranylcypromine and pargyline on release, uptake, and metabolism of noradrenaline in rat brain have been examined in detail after intracisternal administration of tritiated amine. The content of labelled amine remaining in the brain is determined by the initial neuronal uptake as well as subsequent release and metabolism. If a drug is administered after the amine, its efforts on release and metabolism can be studied; if a drug is given before the amine and the animal is killed within 5 or 6 minutes thereafter, its effects on initial neuronal uptake can be determined. Using this technique, tranylcypromine and pargyline have been shown to produce an increase in the tritiated O-methylated metabolite (normetadrenaline), and a large decrease in tritiated deaminated (3,4-dihydroxymandelic acid) and deaminated O-methylated (3-methoxy-4-

hydroxymandelic acid) metabolites of noradrenaline. Tranylcypromine also decreased the initial uptake of labelled noradrenaline and released some of the accumulated amine, whereas pargyline did not (Schildkraut, 1970).

The mechanisms described above all involve an action on the brain transmitters directly. Other theories have been postulated which are concerned with amine precursors. MAO inhibitors preserve a number of amines which are intermediates in the synthesis of catecholamines, and these intermediates could either 1) displace noradrenaline from its binding site and cause more to be present at receptors, or 2) interfere with the action of noradrenaline at the receptor causing a decrease in its effectiveness (Biel, 1968). This latter postulate is linked to the theory that depression is the consequence of a severe anxiety state (Davey et al., 1963), and antidepressants have a sedative action on postulated anxiety centers (Hare, 1962). It is also possible that the precursors could act as false transmitters and themselves produce an anti-anxiety effect upon combination with receptors. While these possibilities cannot be disproven, they represent rather complicated modes of action, and because they are based only on indirect evidence they are not as generally accepted.

F. EFFECTS OF NARCOTIC ANALGESICS ON BRAIN AMINES

In the majority of studies measuring the effect of narcotic analgesics on brain amines, morphine has been the agent

used. The effects of acute or chronic morphine administration are complex, however, and depend on the dose of the drug and the species of animal. Vogt (1954) showed that morphine given acutely lowered noradrenaline concentrations in the hypothalamus and midbrain of the cat but had little effect on the dog brain. This observation in cats was confirmed by Quinn et al. (1958), but they found no effect in rabbits. An extensive study of the effects of morphine was carried out by Gunne (1963) who found that acute administration lowered brain noradrenaline in rats and cats. Levels tended to return toward normal after long term treatment in cats and to increase in rats. The MAO inhibitor nialamide caused a greater increase in noradrenaline in morphine-tolerant rats than in controls. 5-Hydroxytryptamine was not affected at any time.

Attempts have been made to correlate morphine analgesia with changes in levels of brain monoamines. Compounds such as reserpine, which lower amine levels, generally produced a decrease in morphine analgesia (Schneider, 1954). Administration of p-chlorophenylalanine, which decreases 5-hydroxytryptamine levels by inhibiting synthesis of the amine, also decreased analgesia due to morphine (Weissman, 1967). Takagi et al. (1964) found that the antagonism of morphine analgesia by reserpine and tetrabenazine could be decreased by the previous administration or the precursors 5-hydroxytryptophan and DOPA, which tended to restore amine levels to normal. Previous administration of MAO inhibitors was shown to potentiate the

analgesic property of morphine (Gupta, Kulkarni, 1966, Contreras et al., 1969). Although these facts suggest an involvement of central amines in the production of analgesia, the exact mechanism is still unknown.

Information regarding other analgesics and amine changes is scarce. Levorphanol was shown to have no effect on noradrenaline levels in the rat during chronic administration (Akera and Brody, 1968). A single injection of meperidine (25 mg/kg) given intraperitoneally to rats produced an increase in brain 5-hydroxytryptamine when measured 25 minutes later (Bonnycastle et al., 1962). No other information on meperidine could be found, and the effects of this compound on brain amines would not necessarily be expected to resemble those of morphine.

II. METHODS

METHODS

A. GENERAL PROCEDURE

1. Solutions

The procedures used in preparing drugs, amine standards and reagents described in these methods are listed in the Appendix. All solutions were stored in glass bottles, as fluorescent contaminants can be absorbed from plastic.

2. Glassware

Glassware was thoroughly washed with detergent (Sparkleen, Fisher Scientific Co.), rinsed four times with hot tap water, and then rinsed three times in distilled, deionized water. Extensive rinsing was necessary to remove all traces of detergent, as Sparkleen was found to be highly fluorescent at the wavelengths used for both catecholamines. Periodic comparisons were made between the residual fluorescence from glassware cleaned in concentrated nitric acid, and from glassware cleaned in detergent, and there was no difference.

3. Animals

Male Sprague-Dawley rats weighing between 300 and 400 g were used in all experiments. The animals were purchased from Canadian Breeding Farms, St. Constant, Quebec. They were housed in groups of 6, fed a standard diet of laboratory rat pellets, and had free access to water.

B. EFFECTS OF PHENELZINE AND MEPERIDINE ON RECTAL TEMPERATURE

During studies of drug-induced changes in body temperature, animals were restrained in special cages designed in this laboratory. The cages had a rectangular base and a semi-cylindrical transparent plastic top which closely fitted the body of the animal. One end of the cage had a 2 by 6 cm vertical opening through which the tail protruded, allowing easy insertion of a thermistor into the rectum. The rectal temperature was monitored with a digital readout thermosensor, constructed by H. Brown of this department. A plot of the digital readout versus temperature is shown in Figure 3. The curve is linear between 32 and 45°C, permitting the use of this instrument for accurate recording of small temperature changes. Rats were removed from the cage for injection. Drugs were administered in pyrogen-free saline in a volume of 0.1 ml per 100 g of body weight and all injections were given intraperitoneally.

C. EFFECTS OF PHENELZINE AND MEPERIDINE ON BRAIN AMINES

1. Injection and killing of animals

Rats were divided into groups of three or six animals. Rats within each group received identical doses of drugs for the same pretreatment time. Before an experiment each animal was weighed, and labelled with a code on the dorsal aspect of the tail. This permitted the subsequent injection of all animals within a minimal time period. As the length of pretreatment varied, injection times were adjusted so that all

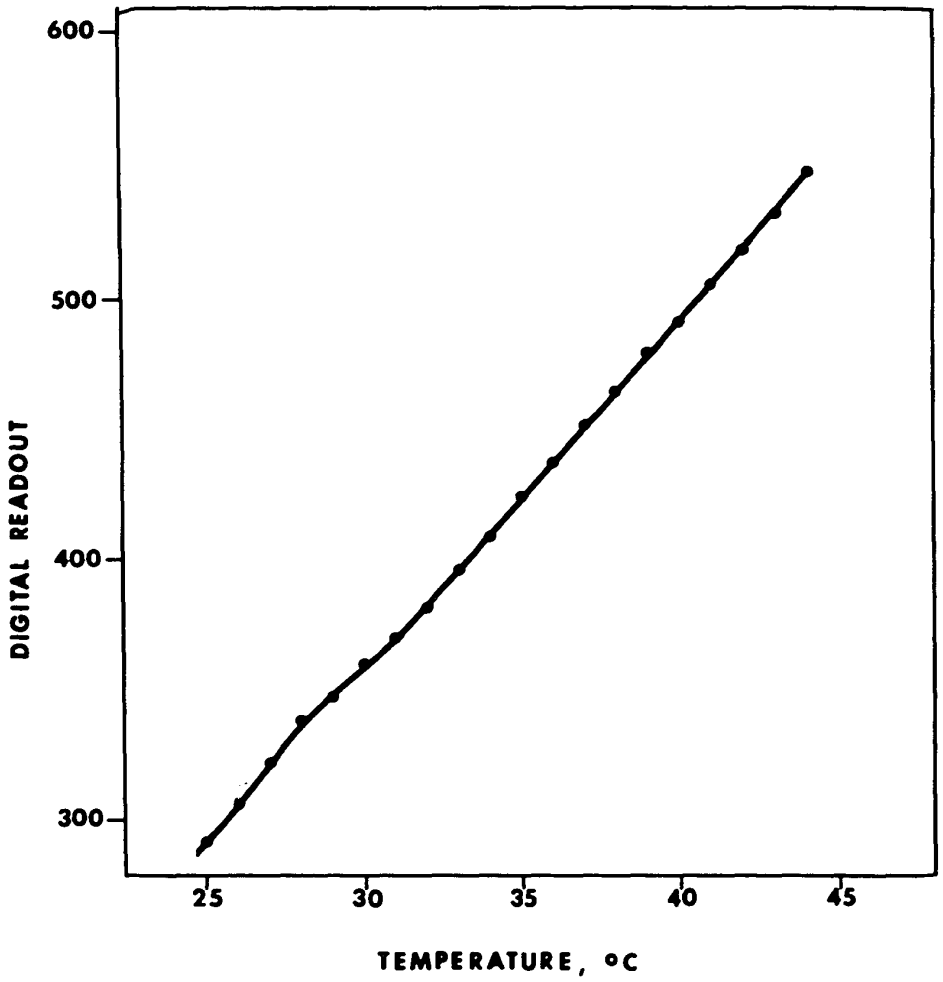


Figure 3: Calibration curve for the digital readout thermosensor: a plot of digital readout versus temperature.

rats were killed between 1 and 2 p m. This was done in an attempt to minimize variations caused by the circadian rhythm in brain monoamine which has been observed by other workers (Dixit and Buckley, 1967; Quay, 1968; Manshardt and Wartman, 1968). Drugs were administered intraperitoneally in a volume of 0.1 ml per 100 g of body weight, and the time of injection was recorded for each animal. In each experiment a group of control rats was injected with isotonic saline, 0.1 ml per 100 g of body weight.

At the appropriate time each rat was killed by dropping it, head first, into a large insulated tank filled with liquid nitrogen, -196°C. This provided three advantages over traditional methods. 1) Acute stress has been shown to alter the metabolism in rat brain of all three amines under study (Bliss et al., 1968). Welch and Welch (1968) have shown in mice that substantial changes in amines may occur after only a few minutes of disturbance. Using the technique described above, the rat could be removed from its cage and killed within ten seconds thus minimizing stress-induced alterations in brain amines immediately before death. 2) Postmortem changes in amines (Bertler and Rosengren, 1959) were also avoided as the animals were left in the liquid nitrogen for approximately 15 seconds, which was sufficient to freeze them throughout. They were then stored frozen at -20°C until dissection. 3) Because it was rapid and easy to perform, the method allowed efficient handling of up to

70 rats in one experiment.

2. Dissection and storage of tissue

Dissection of the frozen animals was performed within 24 hours after killing. The code on the tail of each rat was checked at this time to ensure proper identification. The skulls were opened with an electric saw (Model 2 Moto Tool, Dremel Mfg. Co., Montreal). A T-shaped incision was made, intersecting at the foramun magnum, and the two sides of the skull were peeled laterally with forceps. The whole brain was then lifted out with a small spatula and placed in a beaker immersed in crushed ice. The brains from each group of animals (three or six rats which had received identical treatment) were pooled in one beaker and weighed to the nearest mg. They were then homogenized while frozen in 10 ml of acidified butanol reagent per g of tissue, using a Sorvall Omni-Mixer at 70% of full speed for 45 seconds. Both the homogenizer bucket and butanol reagent were kept in the freezer until immediately before use, to avoid destruction of amine due to local overheating during homogenization (Callingham and Cass, 1963). The butanol-brain homogenate was then transferred to refrigerated 50 ml tubes which were tightly stoppered, and it was kept frozen until extraction. Tissue stored in this way has been shown by Ansell and Beeson (1968) to retain its amine content for at least a month, and no apparent loss was observed when samples were divided and analysed at two-month intervals in this laboratory. It was not necessary to store homogenate for this

length of time however, as all samples could be measured within 4 weeks.

3. Formation of fluorescent catecholamine derivatives

Catecholamines were determined using the method described by Ansell and Beeson (1968), which is a modification of the method of Chang (1964). The procedure results in the formation of highly fluorescent hydroxyindole derivatives of noradrenaline and dopamine, which have been used reliably in amine assays since the work of Lund in 1949. Iodine is used as the oxidizing agent to form quinone derivatives. In alkaline solution these undergo intramolecular rearrangement to yield cyclicized hydroxyindoles, which are then stabilized by the addition of acetic acid. The formation of noradrenolutine is shown in Figure 4. Dopamine, an amine that does not possess the 1-hydroxy group, undergoes a similar reaction to form a dihydroxyindole.

The same procedure was used to study the fluorescence characteristics of catecholamine derivatives and to measure amines extracted from tissue. In the former experiments, known amounts of amines were added to phosphate buffer, pH 6.5, and in the latter experiments amines were extracted into this medium from brain. One ml of phosphate buffer at pH 6.5, containing the amines, was added to each of a series of 5 ml test tubes. An aliquot of 0.1 ml of EDTA reagent was added to each tube and the mixture stirred on a mechanical vibrator. This served to chelate any contaminating heavy metals. At 5-second intervals,

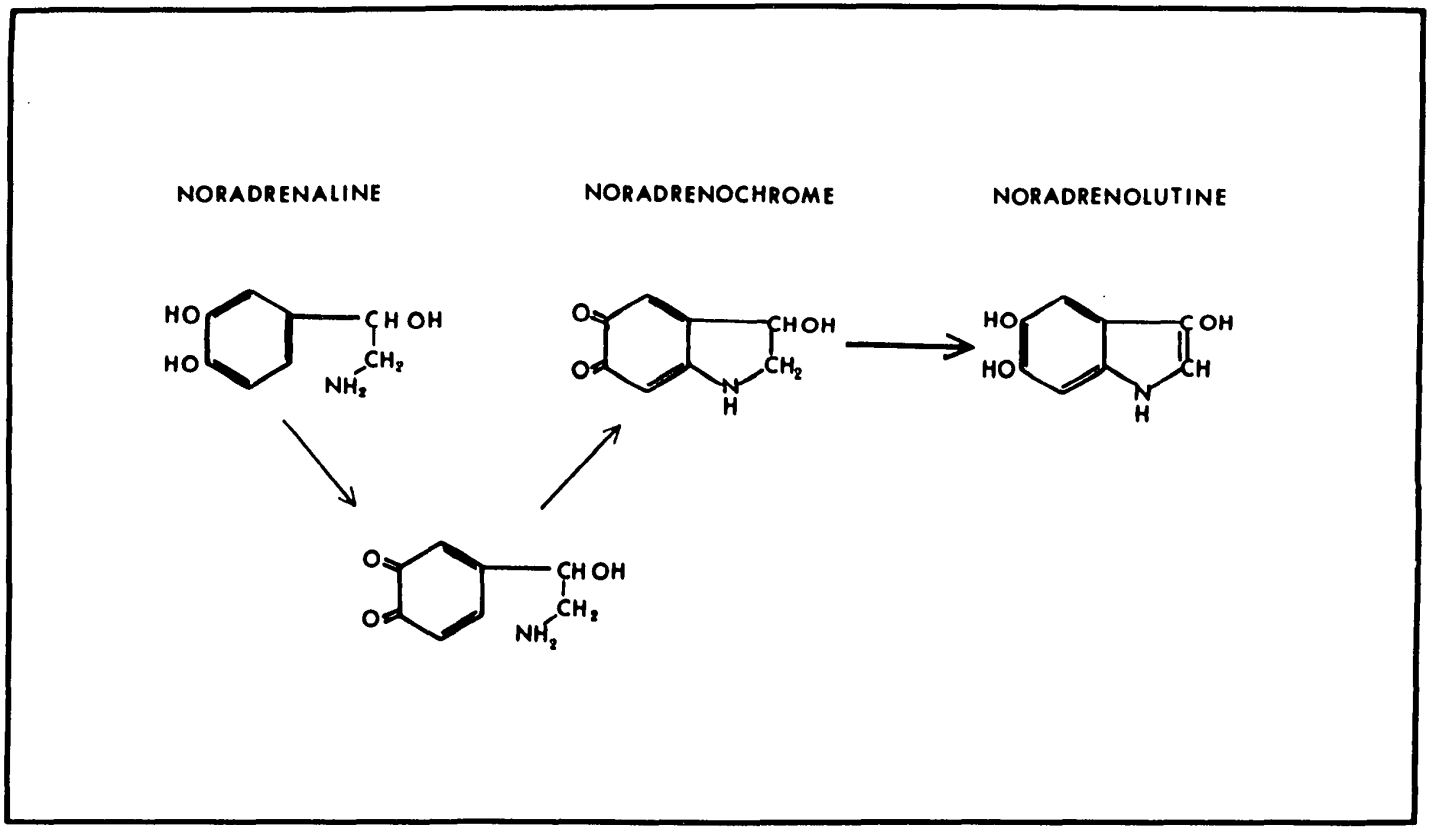


Figure 4: Formation of noradrenolutine from noradrenaline.

0.1 ml of iodine reagent was added from a biopipette to each tube in the series in order. The mixture was stirred on the vibrator immediately after the addition of the iodine. Two minutes later 0.2 ml of freshly prepared alkaline sulfite was added and the mixture was stirred in the same manner, followed by 0.2 ml of 6 N acetic acid 2 minutes later. Each tube was quickly covered, first with a layer of Parafilm (American Can Company, Wisconsin, U.S.A.) and then with a layer of aluminum foil, and the rack of tubes was placed in boiling water. After 2 minutes it was removed and placed in cool water for 15 minutes, then kept at room temperature for 1 hour. Noradrenaline was then measured. Blanks were prepared in a similar manner, except that the phosphate buffer containing the amines was added at the end of the procedure, when the reaction was completed, rather than at the beginning. After measurement of noradrenaline 1.0 ml of the solution was removed from each tube and added to 1.0 ml of distilled deionized water. This series of tubes was covered, mixed and heated in a boiling water bath for 4 minutes and again cooled. Dopamine was measured approximately 10 hours later.

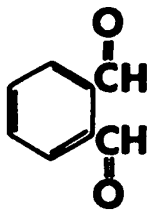
The fluorescent derivatives were measured in an Aminco-Bowman Spectrophotofluorometer, Model 4-8202, equipped with a solid state photomultiplier microphotometer, Model 10-267. Slit widths were set at 5.0 mm, and 1 cm quartz cuvettes were used. Activation and emission wavelengths were 385 mμ and 485 mμ, respectively, for noradrenaline, and 320 mμ and 370 mμ for dopamine. Fluorescence was linearly related to concentration

over the desired range for both catecholamines, as shown in Figures 5 and 6.

When measured under these conditions, the hydroxyindoles were readily distinguished from one another, as a given concentration of either amine produced the same fluorescence in the presence or absence of the other. The difference occurs because the fluorophores of noradrenaline and dopamine have different absorption and emission maxima, different susceptibilities to destruction by heat, and different decay times. Noradrenolutine begins to lose its fluorescence within several hours, whereas the derivative of dopamine is stable for at least 24 hours as described by Ansell and Beeson (1968) and verified in this laboratory. The addition of 5-hydroxytryptamine had no effect on either assay.

4. Formation of a fluorescent 5-hydroxytryptamine complex

5-Hydroxytryptamine was determined according to the method of Maickel et al. (1968), who found that this amine could be reacted with ortho-phthalaldehyde (OPT) under conditions of heat and strong acid to yield a highly fluorescent product.



OPT

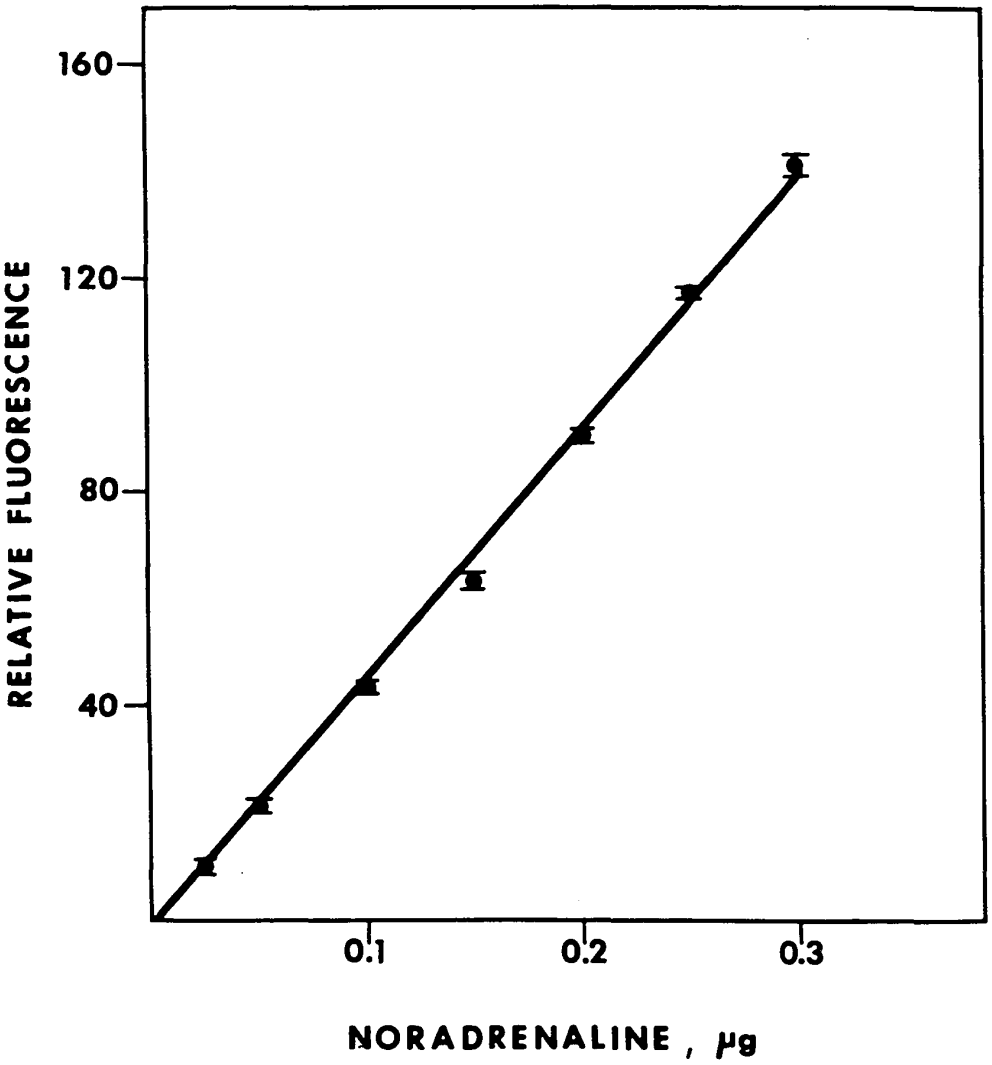


Figure 5: Fluorescence of the trihydroxyindole derivative of noradrenaline.
Each point represents the mean ± SE of 3 determinations.

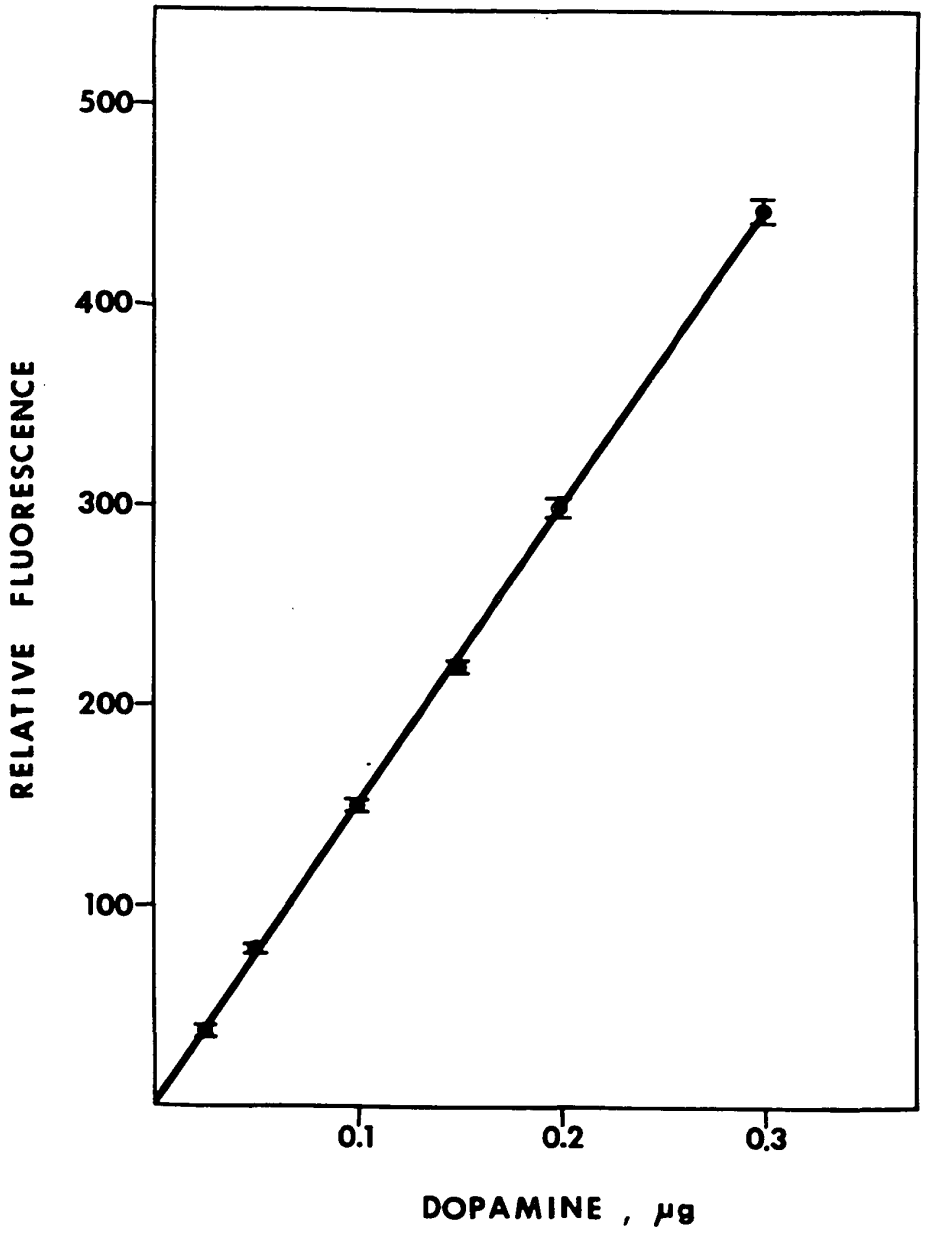


Figure 6: Fluorescence of the dihydroxyindole derivative of dopamine.
Each point represents the mean ± SE of 3 determinations.

Examination of the fluorescence characteristics of 5-hydroxytryptamine and measurement of brain extracts were carried out in the same manner. A volume of 1.2 ml of freshly prepared solution of OPT (4.0 mg/100 ml of 10 N HCl) was added to test tubes containing various amounts of 5-hydroxytryptamine in 0.2 ml of 0.1 N HCl. The tubes were stirred with a mechanical vibrator, covered with Parafilm and aluminum foil, and placed in a boiling water bath. After 15 minutes they were removed and cooled in tap water for 15 minutes, then left at room temperature. Fluorescence was measured one hour later, using activation and emission wavelengths of 360 mμ and 470 mμ respectively, with a slit width of 5.0 mm. Blanks were prepared by adding the amine to the OPT solution immediately before measurement. Relative fluorescence and amine concentration were linearly related, as shown in Figure 7. The addition of either noradrenaline, dopamine, or both of these amines to the original solution had no effect on the resulting fluorescence.

5. Extraction of amines from brain

Monoamines were extracted from brain using a modified organic solvent technique which has been derived from methods described by Chang (1964), Ansell and Beeson (1968), and Maickel et al. (1969). The procedure was divided into three parts.

A. Frozen brain homogenate was removed from the freezer, allowed to thaw, and then thoroughly mixed. A volume of 7.5 ml of the homogenate was added to each of a series of

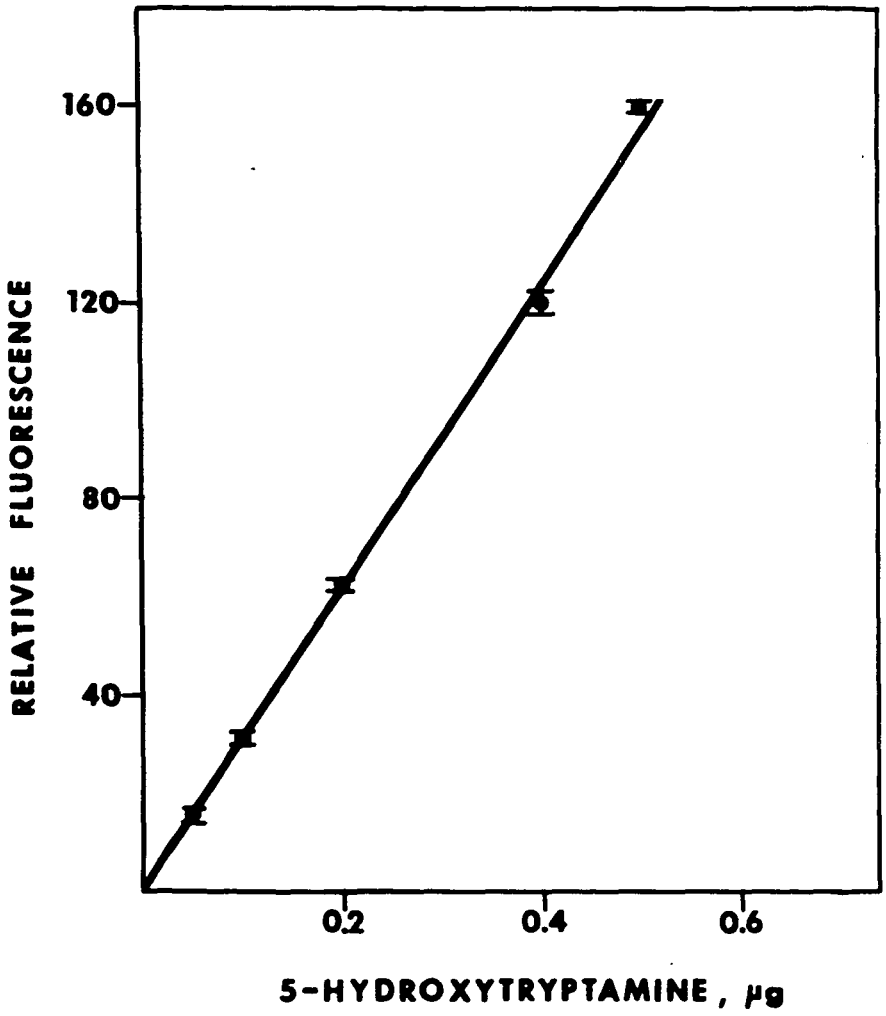


Figure 7: Fluorescence of the 5-hydroxytryptamine-OPT complex. Each point represents the mean ± SE of 3 determinations.

labelled 13 ml test tubes, followed by 0.1 ml of 0.005 N HCl, containing either no amine or increasing concentrations of mixed amine standards. Three or six replicates were made for each concentration of the internal standards. The test tubes were sealed and placed in a mechanical shaker at high speed for five minutes. Following this the tops were removed and the tubes were centrifuged at 400 x g in a Sorvall GLC-1 centrifuge for five minutes. Portions of the supernatant were carried through further procedures while the pellet which formed at the bottom was discarded.

B. Four ml of the supernatant from A was added to 50 ml tubes containing 5 ml phosphate buffer, pH 6.5. Ten ml of isooctane was added to return the amines to the aqueous phase. This mixture was shaken for five minutes and then centrifuged at 400 x g for 5 minutes. The organic layer was removed by suction and discarded along with the tissue debris which collected at the interface. One ml of the aqueous phase was removed from each tube and placed in one of a series of 5 ml test tubes, to be used for the determination of noradrenaline and dopamine, as previously described. The aqueous phases remaining in the first six tubes were retained for the preparation of tissue blanks. For these blanks, 1 ml of the brain extract was added to each of six tubes containing premixed reagents.

The final fluorescence obtained was linearly related to the concentration of internal standard added to the original

butanol phase for both noradrenaline and dopamine. The extractions of catecholamines from butanol reagent and from butanol-brain homogenate were compared. The results for noradrenaline are shown graphically in Figure 8. The results for dopamine are graphically represented in Figure 9.

The extractions were similar for both catecholamines, whether or not the butanol phase contained homogenized tissue. As shown in Figures 8 and 9, the plots of fluorescence versus added amine standard had a similar slope in both cases, indicating that interference with percentage extraction by other brain components was low. Hydrochloric acid, 0.1 N may be used in place of the phosphate buffer as the final aqueous phase in the extraction of catecholamines. A comparison of the two was made, and since linearity between fluorescence and amine standard was greater using phosphate buffer it was used in all procedures. As shown in Figures 8 and 9, variability between identically treated samples of the same homogenate was low.

C. An aliquot of 2.5 ml of the supernatant from A was added to test tubes containing 5 ml isooctane and 0.4 ml 0.1 N HCl. This mixture was shaken for five minutes, centrifuged at 400 x g for 5 minutes, and the supernatant and tissue debris were removed by suction. From the acid phase 0.2 ml was transferred to 20 ml test tubes for the subsequent determination of 5-hydroxytryptamine. The acid phases remaining in the first six tubes were saved to be used for tissue blanks.

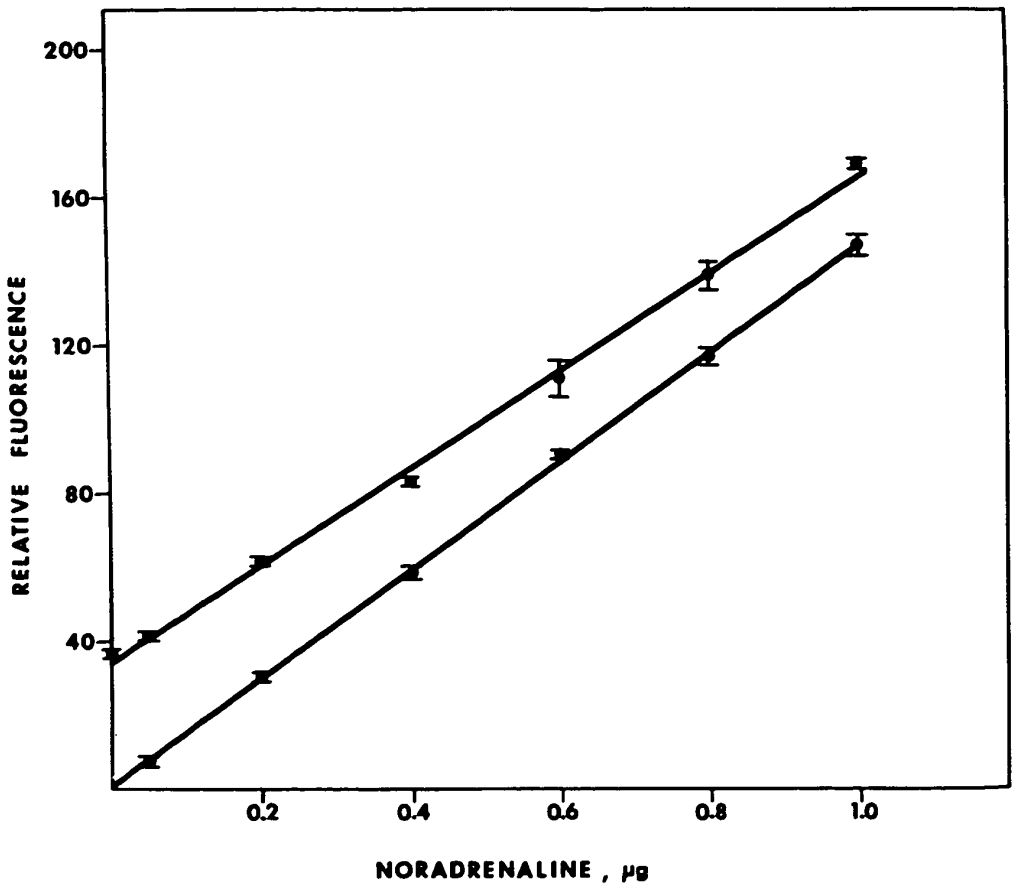


Figure 8: A plot of relative fluorescence versus noradrenaline internal standard, µg.

Upper curve: extraction from butanol-brain homogenate.
Lower curve: extraction from butanol reagent.

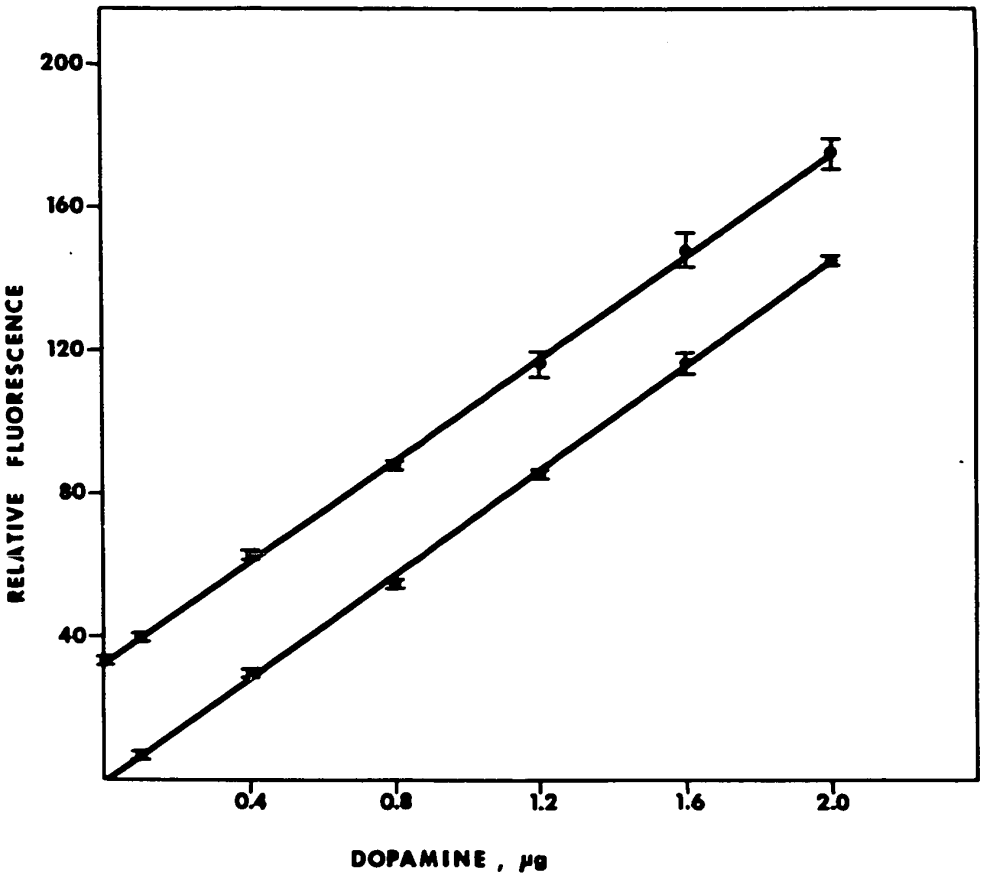


Figure 9: A plot of relative fluorescence versus dopamine internal standard, µg.

Upper curve: extraction from butanol-brain homogenate.
Lower curve: extraction from butanol reagent.

The relationship between amount of internal standard added and fluorescence after extraction was linear, and brain constituents did not interfere with the percent extraction. A comparison of the two extractions is shown graphically in Figure 10.

6. Calculation of tissue amine content

After measurement of fluorescent derivatives in the brain extract alone and in extracts containing increasing levels of internal standards, the tissue blank values (which usually had a range of less than 3% in any experiment) were averaged and the mean was subtracted from each measurement. The means for each concentration were determined and a line was fitted to the series of points by the method of least squares. The Y intercept and slope of the line were then used to calculate the tissue content in $\mu g/g$ in the following manner:

Brain amine content =

$$\frac{\text{Y intercept}}{\text{slope}} \times \frac{\text{volume homogenate/g tissue}}{\text{volume homogenate containing internal standards}}$$

The internal standard system served to minimize variability arising from small daily fluctuations in percent extraction. Tissue levels in normal rats calculated from five initial control experiments, including those recorded in Tables 2, 4 and 6, were 0.54 ± 0.04 $\mu g/g$ of noradrenaline, 1.02 ± 0.11 $\mu g/g$ of dopamine and 0.97 ± 0.06 $\mu g/g$ of 5-hydroxytryptamine. There is considerable variation in the normal levels

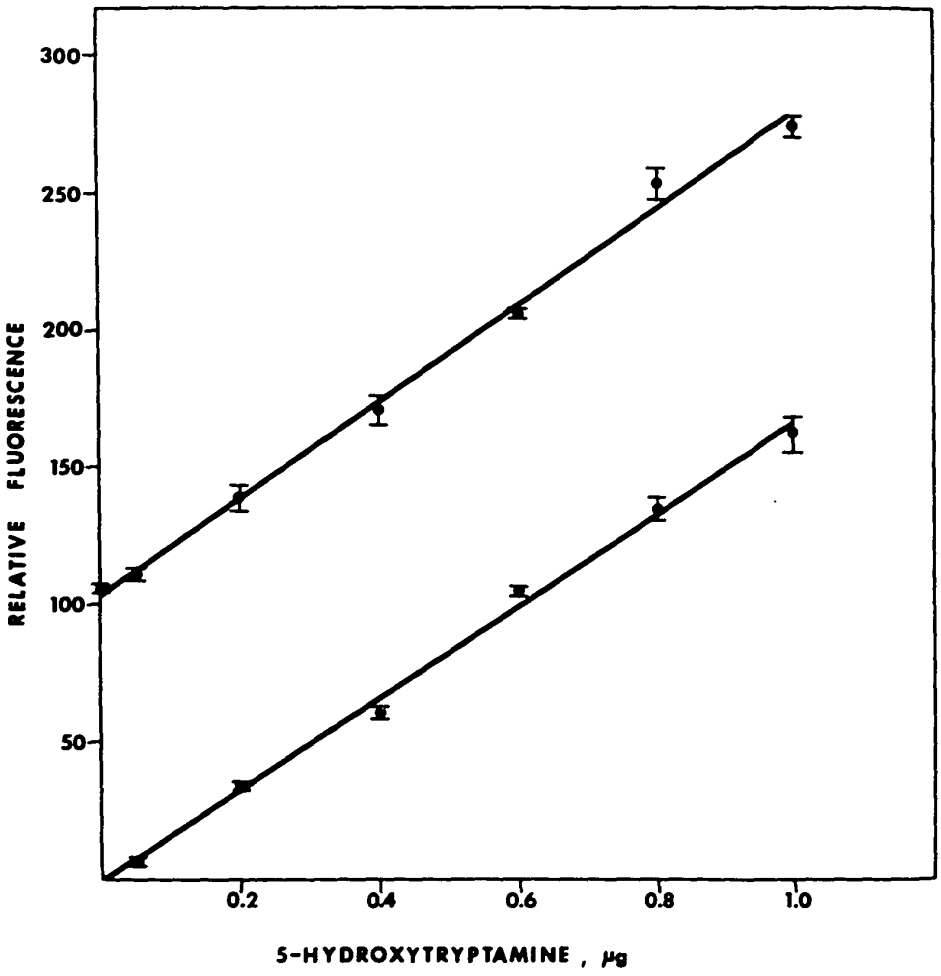


Figure 10: A plot of relative fluorescence versus 5-hydroxy-tryptamine internal standard, µg.

Upper curve: extraction from butanol-brain homogenate.
Lower curve: extraction from butanol reagent.

reported by different workers, however, most found as in these experiments, that absolute amounts of dopamine and 5-hydroxytryptamine were greater than noradrenaline. The values in µg/g for the three amines were within the top part of the range of values given in the literature. Control values in all subsequent experiments and a comparison with those of others is found in Section III (Results). The amounts were calculated using six concentrations of internal standards and six replicates of each. As the curves were linear and the variability low, three internal standards and three replicates of each were considered to provide sufficient accuracy for subsequent studies.

D. STATISTICS

Standard errors of the means and regression lines fitted by the method of least squares were calculated by the methods of Ferguson (1966). The differences between means were tested for statistical significance with Student's t-test. All calculations were performed on an Olivetti-Underwood Programma 101 desk computer, the programs for standard errors, regression lines, and t-tests being retained on magnetic cards.

III. RESULTS

A. EFFECTS OF DRUGS ON ANIMAL BEHAVIOR

An initial step in the study was to confirm that the interaction between phenelzine and meperidine occurred in the strain of rats used. The appearance of representative animals after treatment is illustrated in Figure 11.

1. Phenelzine

Treatment with phenelzine produced little observable response in the rat at doses below 30 mg/kg. Injections of 30 or 40 mg/kg resulted in slight hyperactivity for the first 20 minutes, followed by mild sedation for up to 3 hours. Larger doses (50 to 75 mg/kg) increased the period of sedation to about 6 hours and the animals were notably less responsive to external stimuli.

2. Meperidine

Meperidine, 15 to 25 mg/kg produced a mild Straub reaction, beginning within 3 minutes and lasting for around 20 minutes, and slight sedation. Doses greater than this (40 to 70 mg/kg) caused a more pronounced Straub reaction and, in a few animals, clonic convulsions and death.

3. Phenelzine plus meperidine

Treatment with phenelzine followed by meperidine 6 hours later produced a marked response at all dose levels used. The reaction began within minutes and consisted of a marked Straub reaction (often occurring within 1 minute), arched back, writhing, spread digits, hyperreflexia, and exopthalmos. Clonic

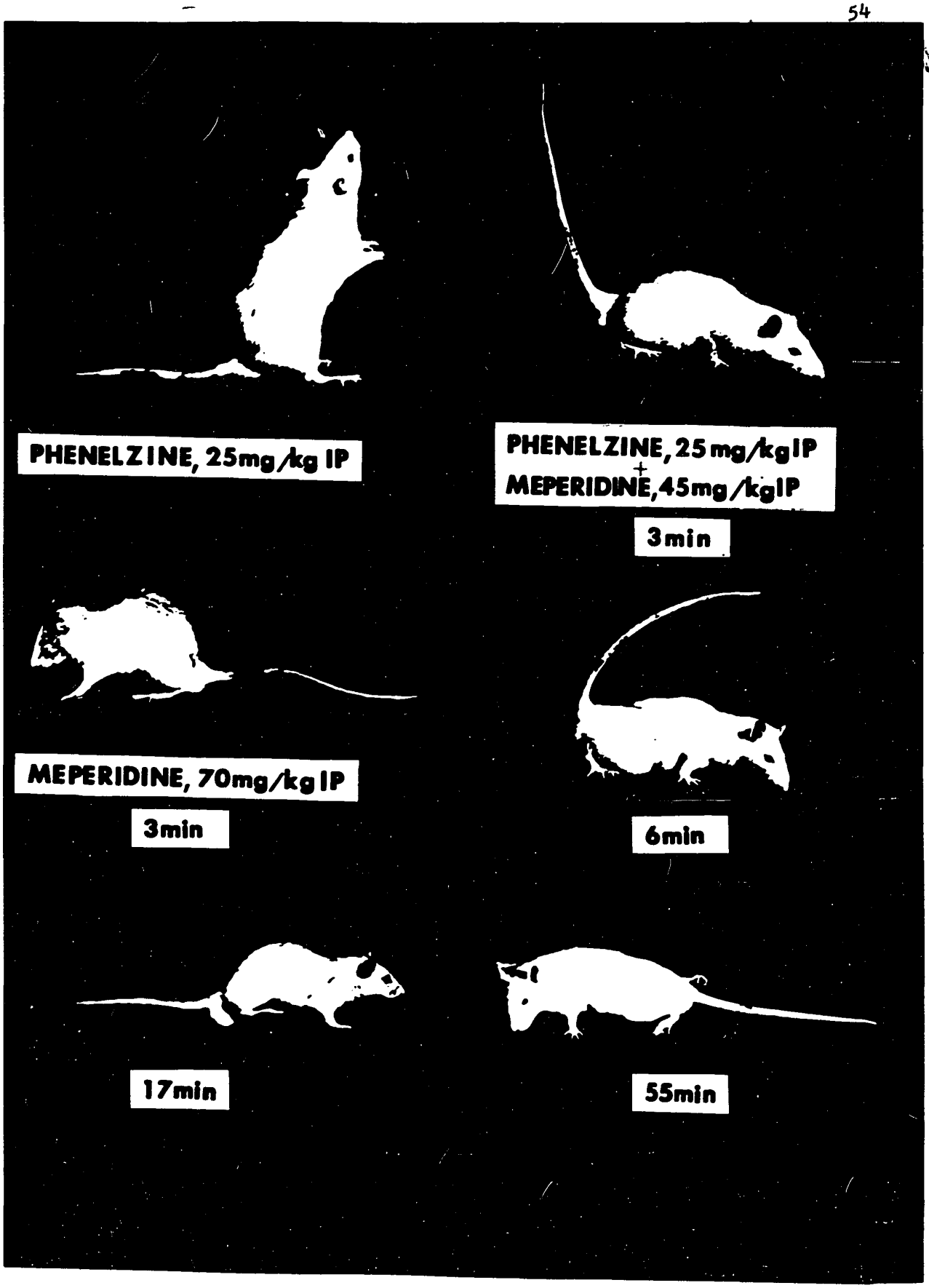


Figure 11: Effects of phenelzine and meperidine on rat behavior.

convulsions occurred in a small proportion of the animals. Symptoms were maximal at around 10 minutes and gradually decreased over a period of 3 hours. A further set of symptoms, occurring only in some of the animals, began at around 10 minutes. Complete muscular relaxation occurred and respiration was markedly depressed with dyspnea and notable cyanosis. These animals either recovered gradually over a period of 3 to 6 hours, or became comatose and died of respiratory depression usually between 15 and 25 minutes.

B. EFFECTS OF DRUGS ON RECTAL TEMPERATURE

Rectal temperature was monitored during the initial examination of the phenelzine-meperidine interaction in rats. Nymark and Nielson (1963) investigated the effect of MAO inhibitors and meperidine on rectal temperature in the rabbit and found that the MAO inhibitor alone slightly increased rectal temperature, meperidine had no significant effect, and the combination of both drugs produced a marked hyperthermia. When death occurred, it was during the phase of maximal temperature elevation. The authors believed that a release or potentiation of brain amines might be involved in this interaction.

1. Phenelzine

Phenelzine significantly lowered body temperature in the rat for up to 4 hours. The results from six animals receiving 25 mg/kg phenelzine are shown graphically in Figure 12. Other doses (40 and 50 mg/kg, three rats per dose) lowered rectal

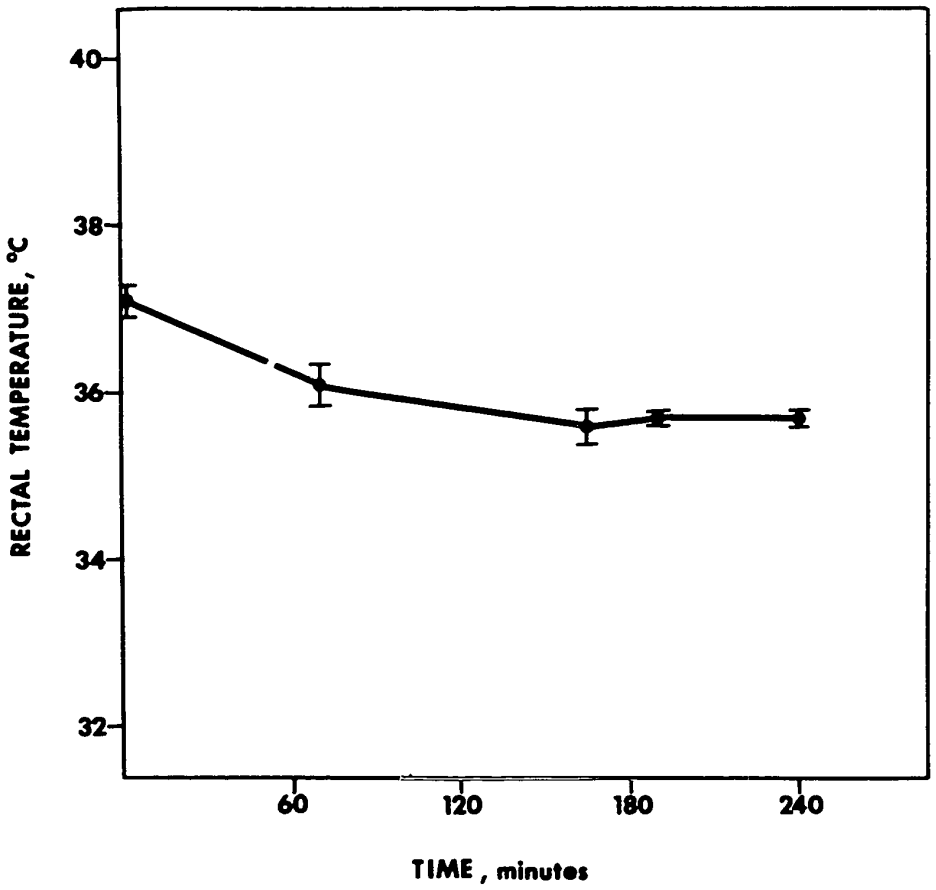


Figure 12: Effect of phenelzine on rectal temperature in the rat.

temperature for up to 6 hours.

2. Meperidine

Meperidine alone had no significant effect on rectal temperature. The effects of 45 mg/kg on four rats are illustrated in Figure 13.

3. Phenelzine plus meperidine

Administration of meperidine (45 mg/kg) after pretreatment for 3 hours with phenelzine produced a further decrease in rectal temperature in all animals, however, the amount of change varied considerably with each rat. After an initial decrease, the temperature in some animals tended to return quickly to previous levels, while in others it continued to drop and the animal died. The rats which died after this combination usually did so within 15 to 25 minutes, at which time hypothermia was maximal. Those animals still alive at 25 minutes usually survived. Figure 14 shows the effect of the phenelzine-meperidine interaction in two representative rats receiving 25 mg/kg of phenelzine and 45 mg/kg meperidine.

That the body temperature decreased during this interaction in the rat, in contrast to the hyperthermia seen in the rabbit, is not surprising, considering the results of other workers who found that identical treatment could increase body temperature in some species and lower it in others (Feldberg and Lotti, 1967). One cannot draw any firm conclusions from these experiments on rectal temperature. They were designed to

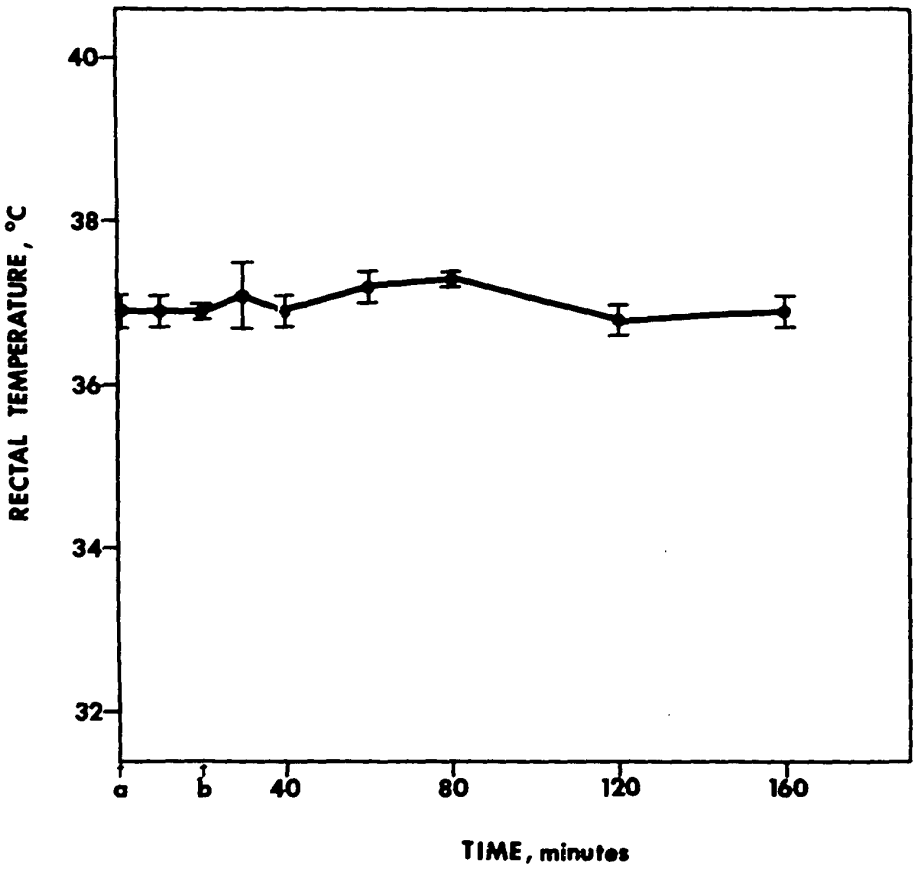


Figure 13: Effect of meperidine on rectal temperature in the rat.

The letters a and b on the abcissa indicate the injection of saline and meperidine, respectively.

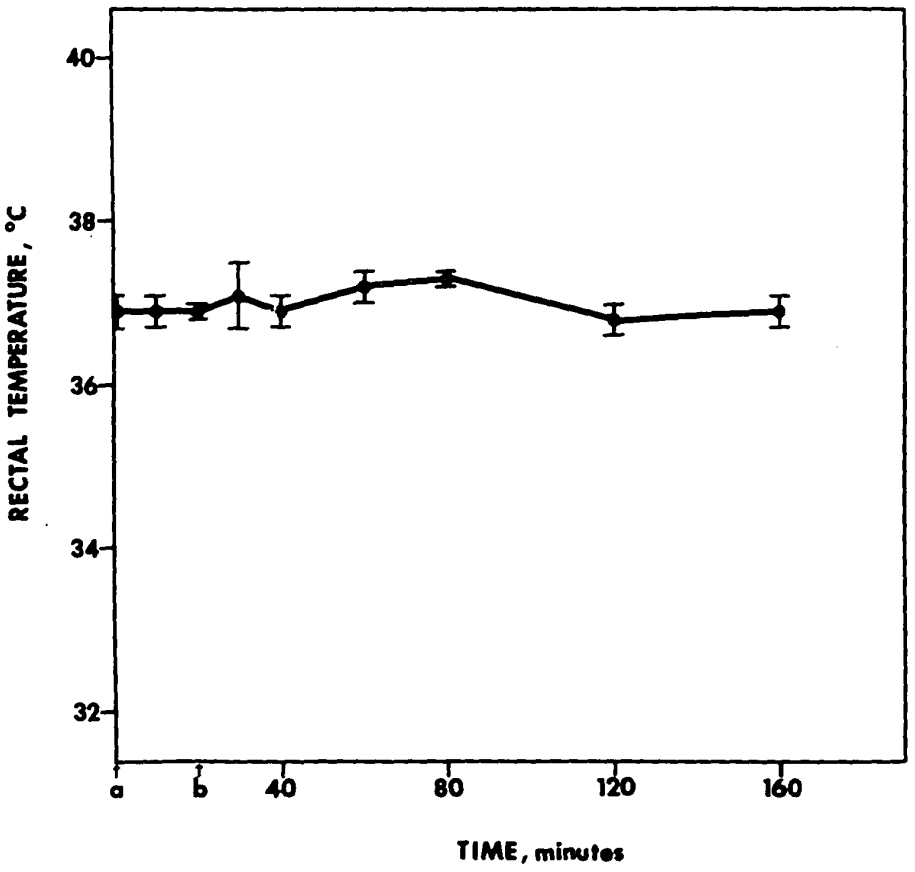


Figure 13: Effect of meperidine on rectal temperature in the rat.

The letters a and b on the abcissa indicate the injection of saline and meperidine, respectively.

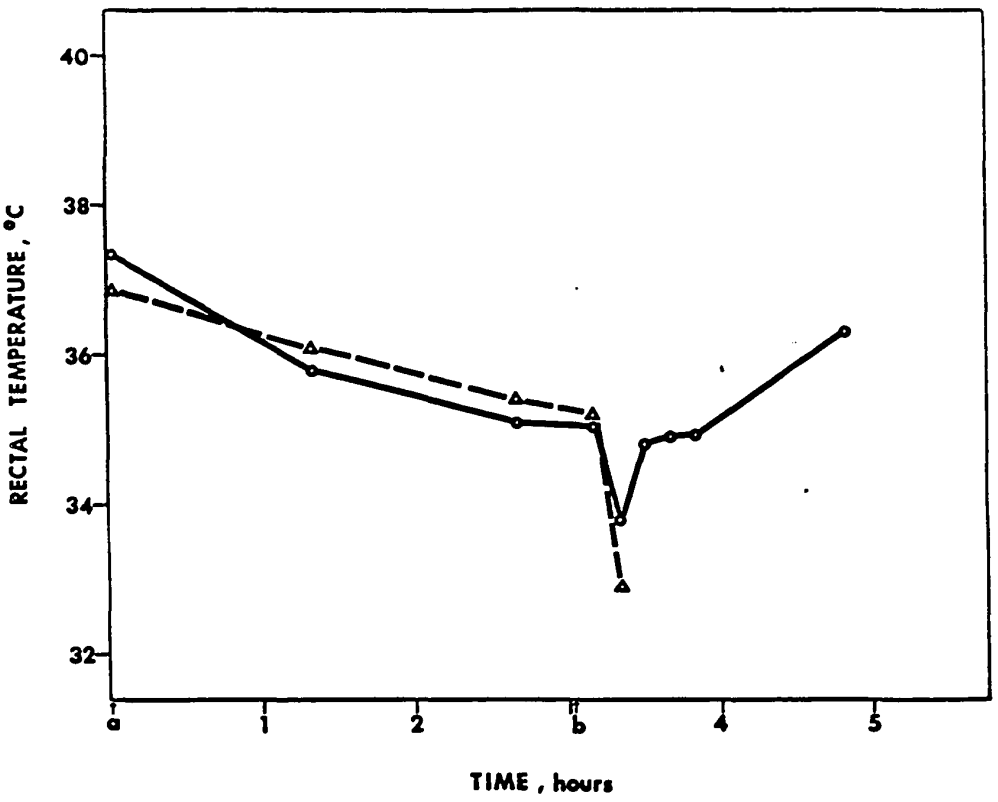


Figure 14: Effects of phenelzine plus meperidine on rectal temperature in two representative rats.

--Δ-- died 15 minutes after meperidine

—o— survived

The letters a and b on the abcissa indicate the injection of phenelzine and meperidine, respectively.

indicate whether or not the phenelzine-meperidine interaction occurred in the rat, and were not intended as an in depth study of the problem utilizing the parameters measured. The results, along with visual observations of behavioral changes, indicated that the interaction did occur in the rat, and this animal would therefore be a suitable model in which to study it further.

C. TOXICITY

1. Phenelzine

During the course of this study 193 rats received phenelzine, in doses ranging from 5 to 75 mg/kg, and no deaths were observed at any time.

2. Meperidine

The LD50 of meperidine in the rat has been estimated at 93 mg/kg ip (Gruber et al., 1941). Doses considerably below this level were chosen in the present study and the lethality observed is outlined below - the number of animals per dose is indicated in brackets.

Meperidine mg/kg	15 (57)	20 (54)	25 (18)	40 (10)	50 (21)
Percent death	5	0	6	20	33

3. Phenelzine plus meperidine

Pretreatment with phenelzine for 6 hours increased the toxicity of meperidine when compared to animals receiving the analgesic alone. The mortality after both drugs was as follows.

Phenelzine mg/kg	10 (15)	20 (20)	30 (20)	25 (90)
Meperidine mg/kg	20	20	20	15
Percent death	0	25	35	11

D. EFFECTS OF DRUGS ON BRAIN AMINES

The results from experiments measuring noradrenaline dopamine, and 5-hydroxytryptamine levels in the rat brain are divided into three sections, one for each amine. The absolute values of amines in μg/g brain are recorded in Tables 1-12. Each table contains the individual values and means ± standard errors (SE's) from a group of experiments. All individual amine levels reported in this study were determined from a pool of six brains taken from identically treated rats, except for the values in Tables 2, 6 and 10, for which a pool of three brains was used. All experiments within a group were done consecutively, to minimize any seasonal variations in brain amine levels of normal rats (Montagu, 1956). The mean values in μg/g have been converted to a percent change, taking control levels as 100%, and these values are shown graphically in Figures 15-23. The results have been expressed in this way for three reasons.

(1) Control values were determined separately for each experiment and it was hoped that expression of the results as percent change from control would allow a reliable comparison between groups of experiments conducted at different times of

the year. (2) Different amines examined have different values in the normal rat and this allowed a comparison between the effects of a drug on noradrenaline, dopamine and 5-hydroxy-tryptamine. (3) The values in µg/g for any amine in the rat brain have been shown to vary with the sex, strain (Quay, 1968), diet (Quay, 1963), and environmental conditions of the animal (Miller et al., 1968), as well as the methods used for extraction and biochemical analysis of tissue (Fleming et al., 1965; Anton and Sayre, 1968; Welch and Welch, 1969). These were kept constant during this series of experiments, but expressing the results in this form allowed a comparison with the results of other workers who might have different control values due to the factors mentioned above.

1. Noradrenaline

The mean control values of noradrenaline were reasonably consistent throughout all experiments, ranging from 0.51 ± 0.03 to 0.59 ± 0.07 µg/g. Miller et al. (1968) compared brain amine levels in male Sprague-Dawley rats obtained from four different breeders, and found that while animals from one source had reasonably consistent levels, intra-strain differences existed between rats from different suppliers. Control levels in the four groups were 0.48 ± 0.05, 0.54 ± 0.07, 0.60 ± 0.07 and 0.67 ± 0.05 µg/g. His techniques for extraction and measurement were similar to those used in this study, and the levels found in the present experiments were acceptable when compared to his results.

a. Phenelzine Groups of six rats were given a constant amount of phenelzine (50 mg/kg) at 3, 6, 12 and 24 hours before death, and their brain amine levels were determined as described in Section II. This time range was chosen because the effects of MAO inhibitors on brain amines have been shown to develop slowly (Brodie et al., 1959) and maximal changes occur at different times in different species (Pletscher, 1966). In these experiments, phenelzine significantly increased noradrenaline levels in the rat brain throughout a 24-hour period, with a maximal elevation occurring 6 hours after administration (Table 1). The mean percent increase with time is shown graphically in Figure 15, and the maximal rise seen at this dose was 64%. Results from a single experiment where groups of 6 animals were killed at 1.5, 4.5, 9 and 18 hours showed an increase of 6, 43, 61 and 56% respectively, confirming the previous results in which 6 hours was the time of maximal effect.

The response to increasing doses of phenelzine at a constant time of 6 hours was then determined, and the amine levels from groups of three rats receiving 10 to 40 mg/kg are summarized in Table 2. All doses produced a significant increase, when compared to control. Figure 16 shows the percent increase with dose, the value for 50 mg/kg from the previous experiment is included. Noradrenaline levels increased with larger doses until 40 mg/kg, and no further increase, in fact a small decrease occurred with 50 mg/kg. This may indicate the beginning of a plateau, as it has been shown that an approximate steady-state

Pretreatment Time, hours	Noradrenaline, µg/g				
	1	2	3	Mean ± SE	% Increase
	0.54	0.45	0.54	0.51 ± 0.03	
3	0.69	0.60	----	0.65 ± 0.04	28.7*
6	0.85	0.81	----	0.83 ± 0.02	64.35*
12	0.74	0.81	0.89	0.81 ± 0.04	60.4*
24	0.66	0.79	----	0.73 ± 0.06	43.6*

TABLE 1: Effect of phenelzine on noradrenaline levels in whole rat brain at various times after pretreatment. Each value is derived from a pool of 6 brains.

* p less than 0.025

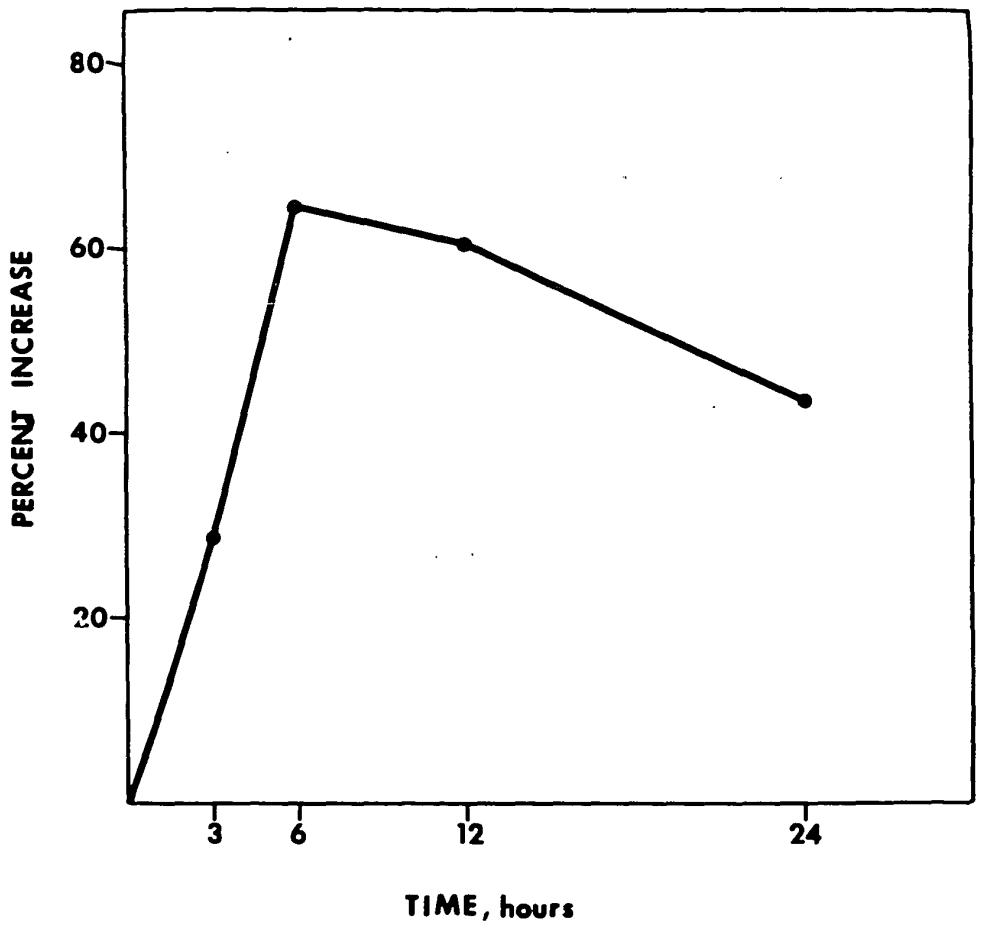


Figure 15: Percent increase of noradrenaline in whole rat brain at various times after the administration of phenelzine.

Phenelzine mg/kg	Noradrenaline, µg/g						
	1	2	3	4	5	Mean ± SE	% Increase
0.0	0.51	0.74	0.58	0.59	0.53	0.59 ± 0.04	----
10.0	0.72	0.66	0.83	0.62	0.71	0.71 ± 0.03*	20.3
20.0	0.63	0.83	0.80	0.85	----	0.77 ± 0.05*	31.0
30.0	0.92	0.97	0.86	0.71	0.90	0.87 ± 0.04*	49.2
40.0	1.64	0.74	1.05	0.95	0.75	1.03 ± 0.16*	74.6

TABLE 2: Effect of increasing doses of phenelzine on noradrenaline levels in whole rat brain. Each value is derived from a pool of 3 brains.

* p less than 0.05.

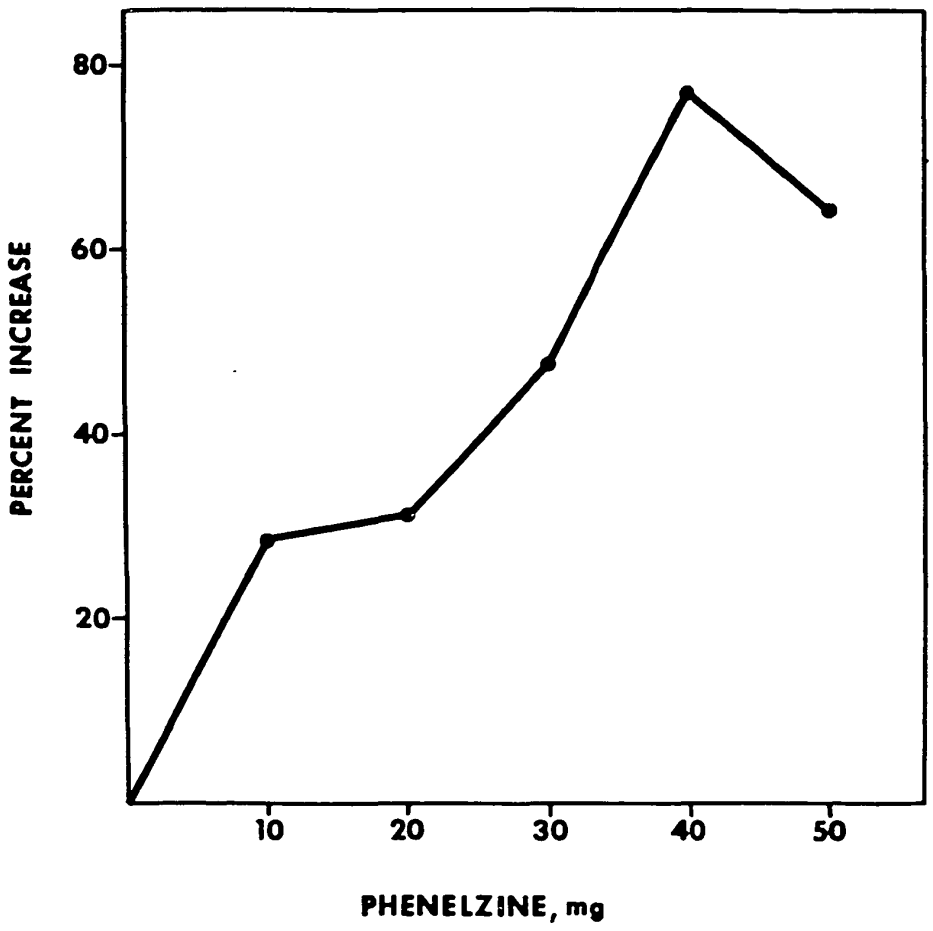


Figure 16: Percent increase of noradrenaline in whole rat brain with increasing doses of phenelzine.

level is reached after MAO inhibition where larger doses of inhibitor produce no further increase in amines (Green et al., 1962).

b. Meperidine Doses of 10 to 50 mg/kg of meperidine were given to groups of six rats and no significant change was observed at any time between 10 and 120 minutes after any dose. Results from three experiments using 20, 25 and 50 mg/kg are given in Table 3. The shorter pretreatment times used with meperidine were based on the assumption that if it produced an observable effect of its own which contributed to the interaction the effect would be measurable within a short time because the symptoms were strongest within the first hour. Single experiments in which amines were measured at various times again showed no significant effect. At 0, 10, 20, 30, 40, 60 and 120 minutes after meperidine (20 mg/kg), noradrenaline levels in groups of six rats were 0.55, 0.53, 0.59, 0.58, 0.56, 0.61 and 0.57 µg/g, respectively. At 0, 10, 15 and 30 minutes after a larger dose of meperidine (50 mg/kg), levels were 0.57, 0.63, 0.62 and 0.62 µg/g respectively.

c. Phenelzine plus meperidine To study the effect of the two drugs in combination on brain amines, groups of six rats were given two injections 6 hours apart, in one of four combinations. The first injection was either phenelzine or saline, the second was meperidine or saline. The animals were killed between 10 and 60 minutes after the second injection.

Meperidine mg/kg	Pretreatment Time, minutes	Noradrenaline, µg/g			
		1	2	3	Mean ± SE
		0.58	0.60	0.54	0.57 ± 0.02
20	30	0.56	0.57	0.57	0.57 ± 0.01
20	60	0.56	0.69	0.57	0.61 ± 0.04
25	30	0.53	0.53	0.55	0.54 ± 0.01
50	30	0.64	0.63	----	0.63 ± 0.01

TABLE 3: Effect of meperidine on noradrenaline levels in whole rat brain. Each value is derived from a pool of 6 brains.

The 6-hour interval between drugs was chosen because a low dose of phenelzine could be used to produce a significant elevation of amines at the time of meperidine administration. In an initial experiment using a very low dose of phenelzine (10 mg/kg), followed by meperidine (20 mg/kg) in groups of six rats, little change in brain amines occurred, and the symptoms of the interaction were mild. The increase in noradrenaline due to phenelzine alone was 20% (Table 2), 5 minutes after the addition of meperidine it was 19%, and at 15 minutes it was 22%. Larger doses of phenelzine were then used to produce higher amine levels before meperidine was administered. A dose of 20 mg/kg of phenelzine elevated brain noradrenaline 31.0% (Table 2), and the administration of meperidine caused further increases to 37, 87 and 83% at 10, 20 and 30 minutes respectively. When 30 mg/kg of phenelzine was given, noradrenaline rose by 49%, and subsequent administration of meperidine caused the levels to rise to 62 and 97% above normal at 10 and 20 minutes. Mortality intervened in the latter experiment, however, as 35% of the animals died consequently the doses were adjusted to 25 mg/kg phenelzine and 15 mg/kg of meperidine. With this dose combination, amine levels were elevated by phenelzine, and the administration of meperidine produced a marked reaction in all rats, however, most of the animals treated (88%) survived long enough to be used for biochemical analysis. Three experiments were performed with these doses, and phenelzine and meperidine alone were included in each experiment for comparison. The effects of phenelzine,

meperidine, and the combination of both drugs on brain noradrenaline are given in Table 4. Phenelzine produced the expected elevation. The effect of meperidine alone was not significant, but all three mean values were greater than the control. The combination of both drugs produced a further increase over that seen with phenelzine alone at all times between 10 and 60 minutes. As shown graphically in Figure 17, the maximal elevation in brain noradrenaline occurred 30 minutes after the administration of meperidine. This level was significantly greater than that seen with phenelzine alone at the same time. As meperidine alone produced a consistent, although not significant, increase in noradrenaline in these experiments, it is possible that the further increase upon administration of meperidine to phenelzine pretreated rats is an additive one.

2. Dopamine

Dopamine levels in the normal rat brain varied considerably between experiments done at different times of the year; however, the values from consecutive experiments were close. No attempt was made to correlate brain amine content with season. Mean values were between 0.65 ± 0.06 (range 0.55 - 0.84) and 1.48 ± 0.09 µg/g (range 1.38 - 1.65). Ansell and Beeson (1968) reported 1.06 ± 0.07 µg/g of dopamine in rat brain, and Bliss et al. (1968) found 0.66 ± 0.07 to 0.91 ± 0.11 µg/g, but these workers did not comment on any long-term variability in dopamine levels.

Treatment 1 - 6 hours	Treatment 2 - 0 hours	Time of death, minutes	Noradrenaline, µg/g				
			1	2	3	Mean ± SE	% Increase
			0.40	0.71 0.65	0.58	0.59 ± 0.07	
Phenelzine		10	0.92	0.99	----	0.96 ± 0.03*	62
Phenelzine		30	0.94	0.84	----	0.89 ± 0.02*	51
Phenelzine		60	0.93	1.02	----	0.98 ± 0.04*	66
	Meperidine	10	0.72	0.70	----	0.71 ± 0.00	20
	Meperidine	30	0.63	0.68	0.80	0.70 ± 0.05	19
	Meperidine	60	0.63	0.81	----	0.72 ± 0.09	22
Phenelzine	Meperidine	10	0.78	1.09	1.37	1.08 ± 0.17*	83
Phenelzine	Meperidine	20	0.98	1.07	1.04	1.03 ± 0.02*	75
Phenelzine	Meperidine	30	1.01	1.32	1.26	1.20 ± 0.09*	103
Phenelzine	Meperidine	40	0.98	1.22	1.33	1.18 ± 0.10*	100
Phenelzine	Meperidine	60	0.76	1.34	1.12	1.07 ± 0.17*	81

TABLE 4: Effect of phenelzine and meperidine on noradrenaline levels in the whole rat brain. Each value is derived from a pool of 6 brains.

* p less than 0.05.

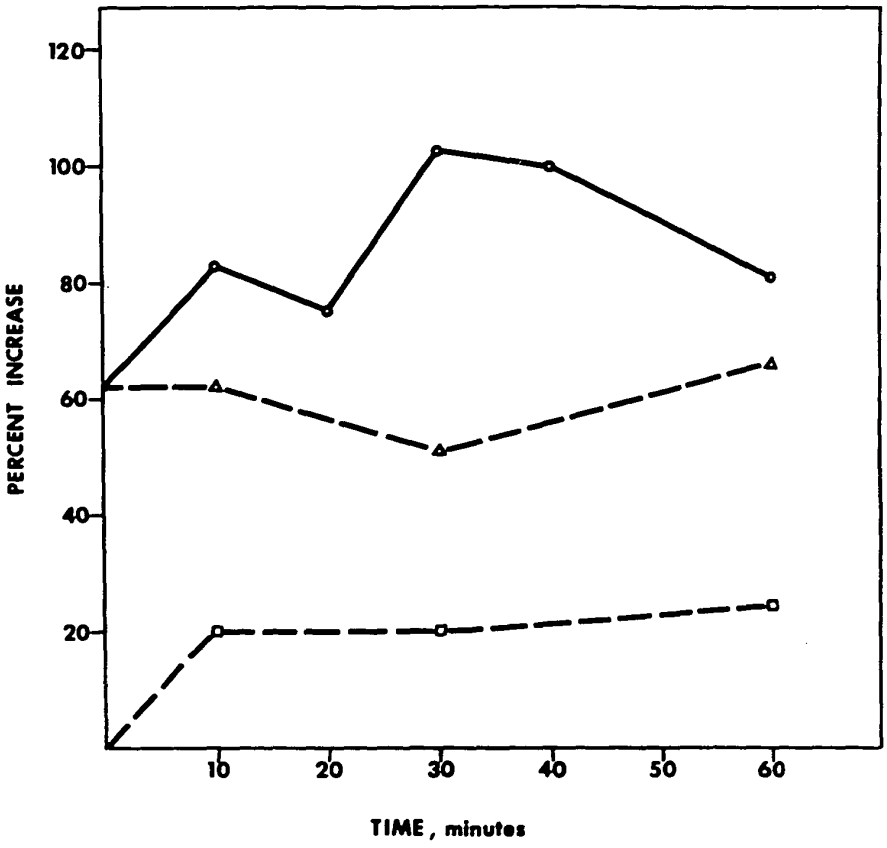


Figure 17: Percent increase of noradrenaline in whole rat brain after phenelzine, meperidine, and phenelzine plus meperidine.

--△-- phenelzine
--□-- meperidine
—○— phenelzine plus meperidine

a. Phenelzine Phenelzine (50 mg/kg) significantly increased brain dopamine levels at 3, 6 and 12 hours after administration. The level at 24 hours was higher than control but this increase was not significant (Table 5). The maximum elevation, as for noradrenaline, occurred at 6 hours. When the effects of phenelzine on the catecholamines were compared, the responses differed notably in two ways. 1) Dopamine increased to a greater extent than noradrenaline. Levels rose to 123% above normal (Figure 18), whereas the maximum elevation seen with noradrenaline was only 64%. 2) The levels of dopamine decreased toward normal rapidly (35% at 12 hours and 22% at 24 hours), while noradrenaline remained high for a longer period of time (43% at 24 hours). A single experiment measuring dopamine at 1.5, 4.5, 9 and 18 hours showed increases of 20, 81, 68 and 30%, confirming the previous results for the time course of phenelzine on brain dopamine.

Increasing doses of phenelzine were administered and dopamine was measured 6 hours later, at the time of maximum response. A low dose of phenelzine (10 mg/kg) produced a small, nonsignificant decrease, whereas all higher doses produced a significant increase in brain dopamine (Table 6). The results are shown graphically in Figure 19, and the percent increase after 50 mg/kg is included from Table 5. The dopamine response seemed to reach a steady state at 30 mg/kg, as doses above this produced no further rise in amine.

Pretreatment Time, hours	Dopamine, µg/g 1	2	3	Mean ± SE	% Increase
	1.65	1.38	1.40	1.48 ± 0.09	
3	2.67	1.86	2.30	2.28 ± 0.23*	54.1
6	2.40	3.20	4.20	3.27 ± 0.52*	123.7
12	2.10	1.94	1.93	2.00 ± 0.05*	35.1
24	2.04	1.59	----	1.81 ± 0.22	22.3

TABLE 5: Effect of phenelzine on dopamine levels in whole rat brain at various times after pretreatment. Each value is derived from a pool of 6 brains.

* p less than 0.025.

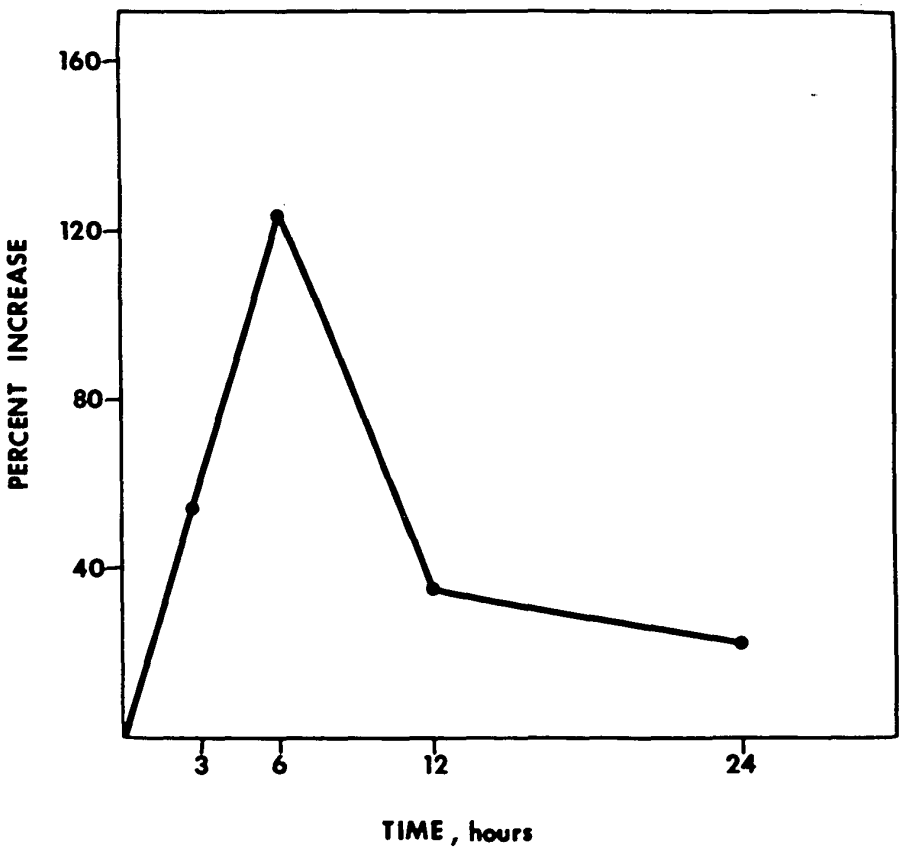


Figure 18: Percent increase of dopamine in whole rat brain at various times after the administration of phenelzine.

b. Meperidine Meperidine did not cause a significant change in brain dopamine at any dose, although a slight increase was observed in each case. Table 7 presents the results of three experiments with different doses at 30 and 60 minutes. Single experiments at different times showed levels of 1.05, 1.16, 1.09, 1.14, 1.60, 1.52 and 1.12 µg/g of dopamine at 0, 10, 20, 30, 40, 60 and 120 minutes after 20 mg/kg meperidine, and 0.97, 1.44, 1.14 and 1.04 µg/g at 0, 10, 15 and 30 minutes after 50 mg/kg meperidine.

c. Phenelzine plus meperidine Rats were given phenelzine, meperidine, or a combination of both drugs as described in Section 1 on noradrenaline. In single experiments where different combinations of doses were used, a low dose of phenelzine (10 mg/kg) decreased dopamine levels by -22.6%. Meperidine (20 mg/kg) slightly elevated this to -13.0 and -18.6% at 5 and 15 minutes respectively. A larger dose of phenelzine (20 mg/kg) produced elevations of 73.1, 41.1, 265.5 and 173.3% at 0, 10, 20 and 30 minutes after meperidine, and 30 mg/kg phenelzine resulted in increases of 122.6, 187.8 and 304.0% at 0, 10 and 20 minutes after meperidine. As mentioned previously, the latter combination proved too toxic, so the levels of both drugs were decreased, and a more detailed examination was performed in three experiments. Phenelzine alone (25 mg/kg) produced a significant increase of dopamine (from 146 to 194% between 6 and 7 hours), and meperidine produced a non-significant increase at 10, 30 and 60 minutes (Table 8). The combination of both drugs caused a marked elevation in brain dopamine at every

Phenelzine mg/kg	Dopamine, µg/g						
	1	2	3	4	5	Mean ± SE	% Increase
0.0	0.51	0.97	0.71	1.58	0.86	0.93 ± 0.18	
10.0	0.38	0.54	1.58	0.54	0.55	0.72 ± 0.22*	-22.6
20.0	0.92	1.45	1.54	1.27	2.88	1.61 ± 0.33*	73.1
30.0	1.73	1.45	2.78	1.79	2.62	2.07 ± 0.26*	122.6
40.0	1.76	1.88	1.45	2.50	----	1.90 ± 0.24*	104.3

TABLE 6: Effect of increasing doses of phenelzine on dopamine levels in whole rat brain. Each value is derived from a pool of 3 brains.

* p less than 0.05.

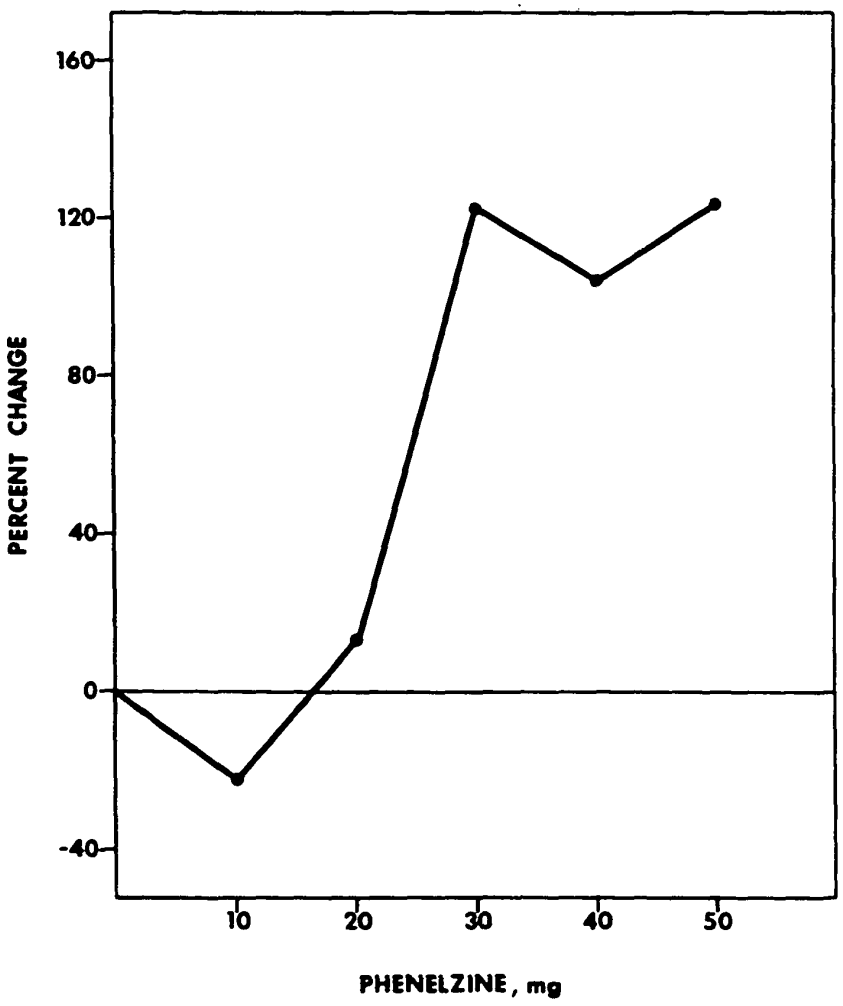


Figure 19: Percent change of dopamine in whole rat brain with increasing doses of phenelzine.

Meperidine mg/kg	Pretreatment Time minutes	Dopamine, µg/g			
		1	2	3	Mean ± SE
		1.07	0.86	1.29	1.07 ± 0.12
20	30	0.88	1.62	2.78	1.76 ± 0.55
20	60	1.52	1.02	----	1.27 ± 0.25
25	30	1.18	1.01	1.14	1.11 ± 0.05
50	30	1.24	1.51	----	1.37 ± 0.14

TABLE 7: Effect of meperidine on dopamine levels in whole rat brain. Each value is derived from a pool of 6 brains.

Treatment 1 - 6 hours	Treatment 2 0 hours	Time of death, minutes	Dopamine, ug/g 1	2	3	Mean ± SE	% Increase
			0.61	0.55 0.59	0.84	0.65 ± 0.06	
Phenelzine		10	2.15	1.20	----	1.68 ± 0.47*	158
Phenelzine		30	1.80	1.40	----	1.60 ± 0.20*	146
Phenelzine		60	1.66	2.15	----	1.91 ± 0.24*	194
	Meperidine	10	0.60	1.65	----	1.13 ± 0.52	74
	Meperidine	30	1.66	0.24	1.13	1.01 ± 0.40	55
	Meperidine	60	0.74	1.22	----	0.98 ± 0.24	51
Phenelzine	Meperidine	10	1.45	1.69	2.13	1.76 ± 0.20*	170
Phenelzine	Meperidine	20	2.37	2.63	1.89	2.30 ± 0.22*	253
Phenelzine	Meperidine	30	3.39	1.05	2.18	2.21 ± 0.67*	240
Phenelzine	Meperidine	40	2.29	4.27	2.15	2.90 ± 0.68*	346
Phenelzine	Meperidine	50	2.90	1.97	3.75	2.87 ± 0.51*	337
Phenelzine	Meperidine	60	1.59	4.35	3.69	3.21 ± 0.83*	394

TABLE 8: Effect of phenelzine and meperidine on dopamine levels in whole rat brain. Each value is derived from a pool of 6 brains.

* p less than 0.05.

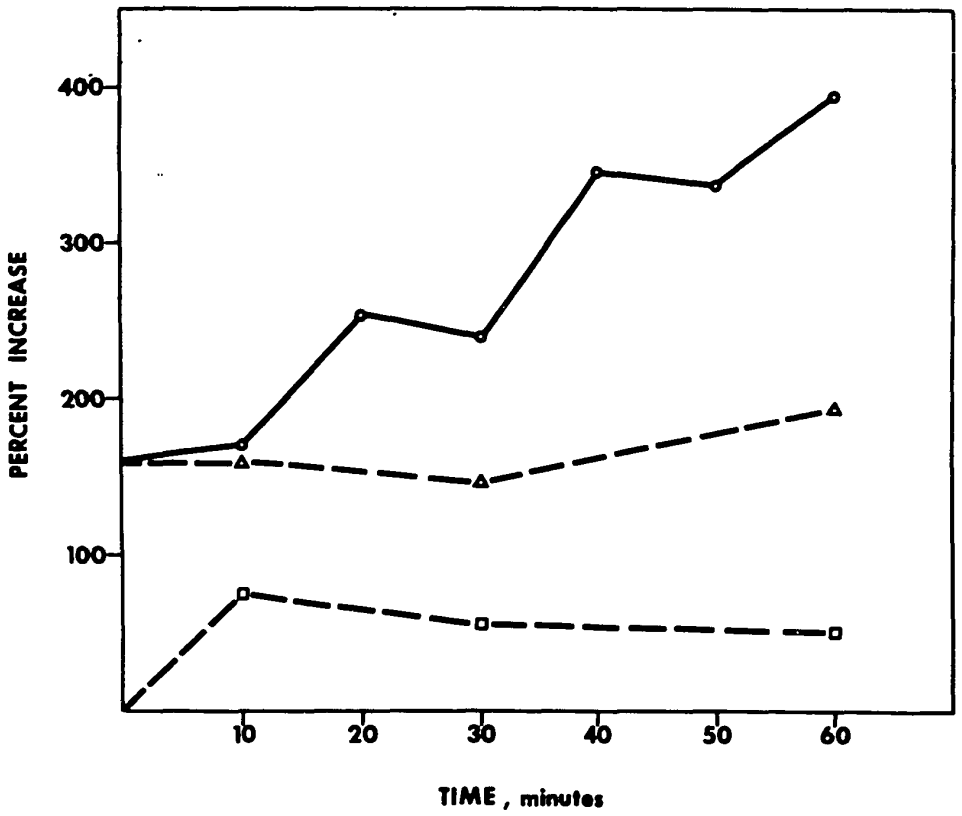


Figure 20: Percent increase of dopamine in whole rat brain after phenelzine, meperidine, and phenelzine plus meperidine.

---△--- phenelzine
---□-- meperidine
——○—— phenelzine plus meperidine

time interval (Figure 20). In this case, dopamine rose to 394% above normal, which was 200% higher than seen with any dose of phenelzine alone. It seemed likely from these experiments that meperidine alone has some effect on brain dopamine which can be compensated for under normal conditions, as a slight (although not significant) increase was seen after meperidine in all experiments (Tables 7 and 8). When meperidine is administered after a MAO inhibitor the combined effect of the two drugs may be involved in the reaction.

3. 5-Hydroxytryptamine

Levels of 5-hydroxytryptamine in the normal rat varied according to the time of the year. No attempt was made to correlate brain amine variations with seasonal changes. Mean values with the ranges observed in consecutive experiments were between 0.80 ± 0.04 (0.73 - 0.84) and 1.50 ± 0.16 (1.22 - 1.95). This variation is comparable to the results of others. The OPT method used to determine 5-hydroxytryptamine was derived from methods described by Maickel et al. (1968) and in one report their control values for 5-hydroxytryptamine in whole brains of Sprague - Dawley rats were 0.50 ± 0.04 and 0.55 ± 0.07 µg/g. In a later report published in the same year, control values ranged from 0.76 ± 0.09 to 0.89 ± 0.05 µg/g in different groups of Sprague - Dawley rats.

a. Phenelzine 5-hydroxytryptamine was significantly elevated for up to 24 hours after the administration of 50 mg/kg

phenelzine (Table 9). The maximal increase was seen 6 hours after administration (140%) and a gradual decline occurred over the next 18 hours (to 53%) as shown in Figure 21. Other times examined in one experiment were 1.5, 4.5, 9 and 18 hours, and percent increases were 56, 101, 90 and 76 respectively, confirming 6 hours as the time of maximal response.

The response to increasing doses of phenelzine showed a different pattern than for the catecholamines (Table 10). Although the increase was significant in all cases, the response was small to low doses (28, 31 and 47% increase for 10, 20 and 30 mg/kg respectively) but rose to 77 and 140% respectively after 40 and 50 mg/kg (Figure 22). A similar difference between the responses of catecholamines and 5-hydroxytryptamine has been observed by others (Green et al., 1962; Dubnick et al., 1959).

b. Meperidine The response of brain 5-hydroxytryptamine to meperidine in three experiments is recorded in Table 11. No significant change occurred at any time, although a tendency toward decreased levels was observed. Other times examined in single experiments were 0, 10, 20, 30, 40, 60 and 120 minutes after 20 mg/kg meperidine, and levels were 1.21, 0.84, 1.11, 1.18, 0.94, 0.98 and 1.19 μg/g respectively. After 50 mg/kg of meperidine, at 0, 10, 15 and 30 minutes, levels were 1.16, 0.72, 1.01 and 0.88 μg/g respectively.

c. Phenelzine plus meperidine In single experiments where the dose of phenelzine was varied from 10 to 30 mg/kg and

Pretreatment Time, hours	5-Hydroxytryptamine, μg/g				
	1	2	3	Mean ± SE	% Increase
	0.84	0.84	0.73	0.803 ± 0.036	
3	1.66	1.62	----	1.64 ± 0.02*	104.0
6	1.87	1.98	----	1.93 ± 0.05*	140.0
12	1.17	1.78	1.45	1.47 ± 0.18*	83.1
24	1.27	1.18	----	1.23 ± 0.04*	52.6

TABLE 9: Effect of phenelzine on 5-hydroxytryptamine levels in whole rat brain at various times after pretreatment. Each value is derived from a pool of 6 brains.

* p less than 0.025.

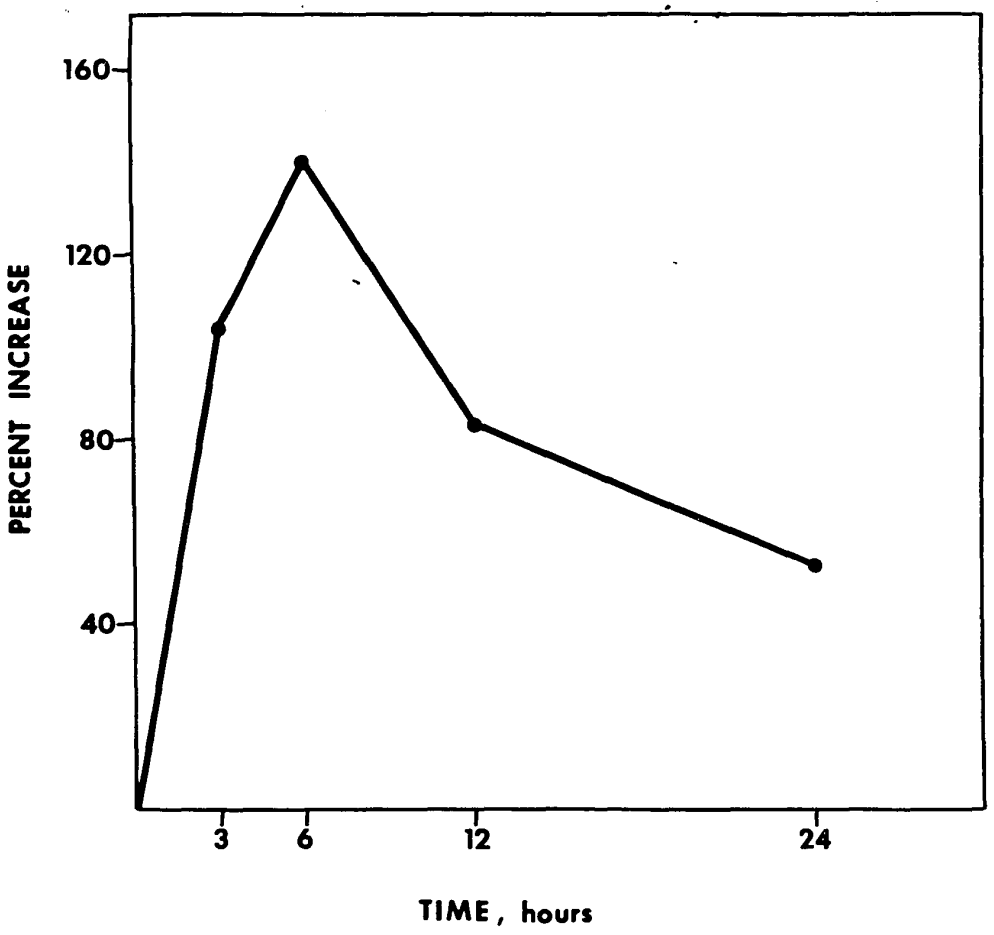


Figure 21: Percent increase of 5-hydroxytryptamine in whole rat brain at various times after the administration of phenelzine.

Phenelzine mg/kg	5-Hydroxytryptamine, µg/g						
	1	2	3	4	5	Mean ± SE	% Increase
0.0	0.995	1.23	1.09	1.18	0.977	1.09 ± 0.05	
10.0	1.43	1.46	1.71	1.28	1.13	1.40 ± 0.10*	28.4
20.0	1.48	1.28	1.35	1.59	1.46	1.43 ± 0.05*	31.2
30.0	1.35	1.65	1.72	1.78	1.54	1.61 ± 0.07*	47.7
40.0	1.62	1.74	2.67	1.65	1.96	1.93 ± 0.19*	77.1

TABLE 10: Effect of increasing doses of phenelzine on 5-hydroxytryptamine levels in whole rat brain. Each value is derived from a pool of 3 brains.

* p less than 0.05.

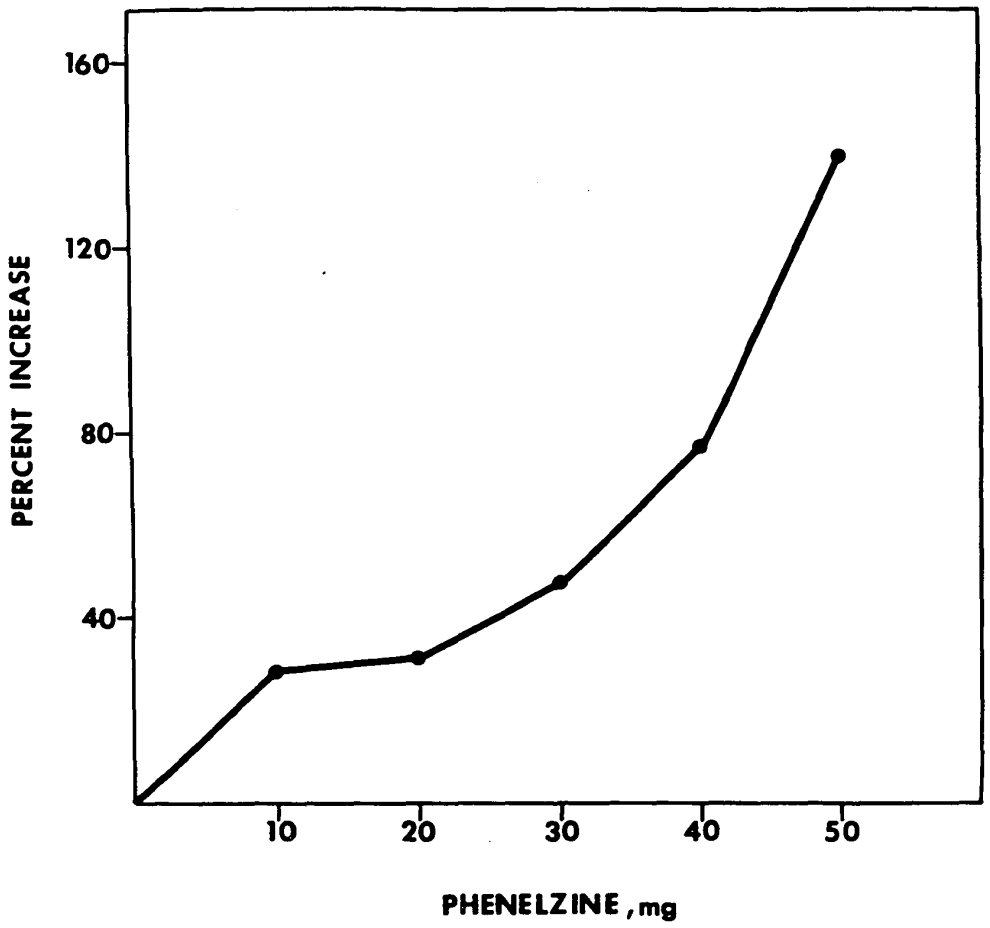


Figure 22: Percent increase of 5-hydroxytryptamine in whole rat brain with increasing doses of phenelzine.

Meperidine mg/kg	Pretreatment Time minutes	5-Hydroxytryptamine, µg/g			
		1	2	3	Mean ± SE
		1.41	1.11	1.30	1.27 ± 0.09
20	30	1.03	1.30	0.76	1.03 ± 0.16
20	60	1.13	0.84	0.89	0.95 ± 0.09
25	30	1.09	1.31	1.38	1.26 ± 0.09
50	30	0.72	1.01	----	0.87 ± 0.15

TABLE 11: Effect of meperidine on 5-hydroxytryptamine levels in whole rat brain. Each value is derived from a pool of 6 brains.

meperidine was kept constant at 20 mg/kg, the percent increases in brain 5-hydroxytryptamine were as follows:

Phenelzine mg/kg	Time after meperidine, minutes 0	5	10	15	20	30
10	28.4	18.9		25.9		
20	31.2		10.0		23.1	13.1
30	47.7		21.6		19.0	

Three experiments were performed using doses of 25 mg/kg phenelzine and 15 mg/kg meperidine. In this series, phenelzine alone produced a significant increase in brain 5-hydroxytryptamine (Table 12), and meperidine produced a small, non-significant decrease at 10 and 30 minutes and a slight increase at 60 minutes. The addition of meperidine to phenelzine-pretreated rats produced a consistent decrease when compared to those animals receiving phenelzine alone (Figure 23).

Treatment 1 - 6 hours	Treatment 2 0 hours	Time of death, minutes	5-Hydroxytryptamine, µg/g 1	2	3	Mean ± SE	% Increase
			1.22	1.41 1.40	1.95	1.50 ± 0.16	
Phenelzine		10	2.60	1.82	----	2.21 ± 0.39*	47
Phenelzine		30	2.75	2.07	----	2.41 ± 0.34*	61
Phenelzine		60	2.11	2.24	----	2.18 ± 0.06*	45
	Meperidine	10	0.75	1.87	----	1.31 ± 0.56	-13
	Meperidine	30	1.43	1.04	1.63	1.37 ± 0.17	- 9
	Meperidine	60	2.01	1.58	----	1.80 ± 0.21	20
Phenelzine	Meperidine	10	1.61	1.78	2.10	1.83 ± 0.14	22
Phenelzine	Meperidine	20	1.75	1.67	2.70	2.04 ± 0.33	36
Phenelzine	Meperidine	30	2.47	1.71	1.64	1.94 ± 0.26	29
Phenelzine	Meperidine	40	1.93	1.73	2.48	2.05 ± 0.22*	37
Phenelzine	Meperidine	50	1.74	1.91	2.24	1.96 ± 0.14*	31
Phenelzine	Meperidine	60	1.47	1.88	2.34	1.90 ± 0.25	27

TABLE 12: Effect of phenelzine and meperidine on 5-hydroxytryptamine levels in whole rat brain. Each value is derived from a pool of 6 brains.

* p less than 0.05.

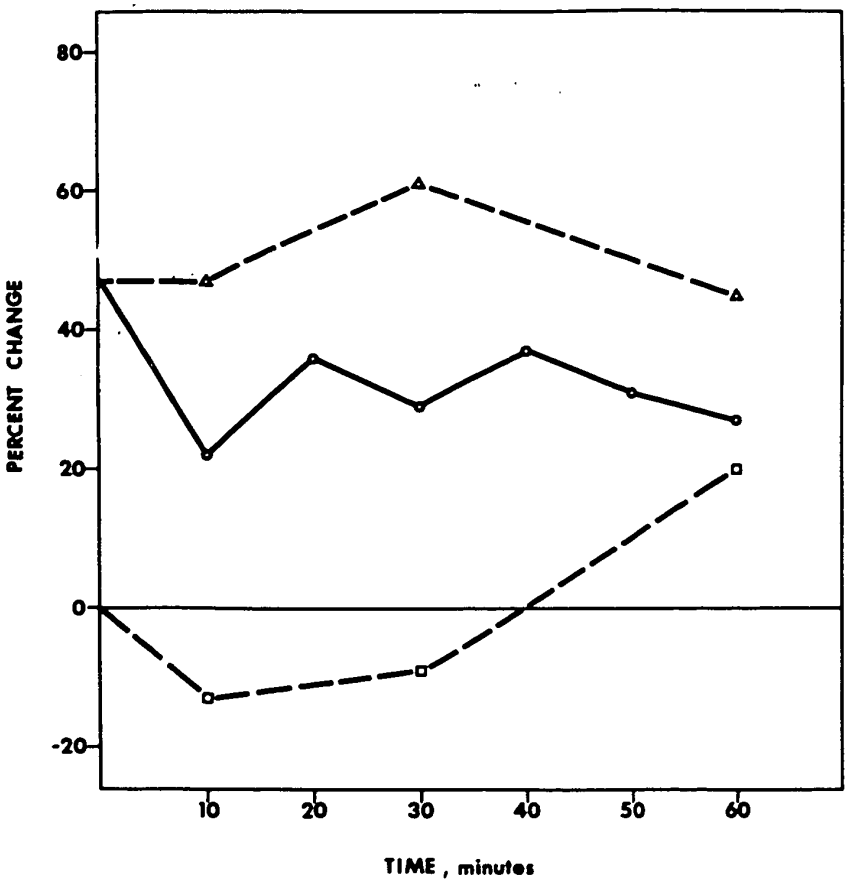


Figure 23: Percent change of 5-hydroxytryptamine in whole rat brain after phenelzine, meperidine, and phenelzine plus meperidine.

--△-- phenelzine
--□-- meperidine
—○— phenelzine plus meperidine

IV. DISCUSSION

A. PREVIOUS STUDIES OF THE PROBLEM

Laboratory investigation on the interaction between monoamine oxidase inhibitors and meperidine began in 1963, when Brownlee and Williams administered 100 mg of phenelzine to mice intraperitoneally and studied the effect on the LD50 of meperidine given four hours later. The toxicity of meperidine increased five fold. Animals became hyperactive within 5 minutes, most deaths occurred within 15 minutes, and those surviving 1 hour recovered. The authors suggested, without any direct evidence, that a decrease in the rate of metabolism of meperidine was responsible and that nalorphine, a specific narcotic antagonist, might be of some use in treating clinical reactions. A similar experiment was conducted on six rabbits (Nymark and Nielson, 1963), using β-p-chlorophenylmercaptoethyl hydrazine as the MAO inhibitor. After 4 days of inhibitor pretreatment, meperidine was administered in doses of 5, 10 and 20 mg per kg using two rabbits per dose. Hyperpyrexia and death occurred in both animals receiving 20 mg per kg, and in one of the two rabbits receiving each of the other doses. Nymark described the symptoms as extreme central stimulation and felt they might be due to a potentiation by meperidine of the action of central amines, known to be elevated by the MAO inhibitor. These results were confirmed by Loveless and Maxwell (1965) using tranyl-cypromine. They also postulated that changes in brain amines might be involved in this interaction but did not study the possibility further.

A comparative study of morphine and meperidine toxicity in mice pretreated with pargyline (150 mg per kg per day) for 5 days showed no decrease in the LD50 for either analgesic when administered 16 hours later. A single dose of pargyline followed my morphine or meperidine in 1 hour increased the toxicity of both compounds equally. This suggests that inhibition of MAO per se was unrelated to the interaction, since maximal inhibition did not occur for several days (Mustala and Jounela, 1966).

Some further information on this problem may be obtained from studies determining the influence of MAO inhibition on narcotic analgesia. The effect of phenelzine on analgesia may differ for morphine and meperidine. Jounela and Mattila (1968) found that in mice phenelzine counteracted morphine analgesia while increasing meperidine analgesia. The toxicity and hypothermic response to both compounds was potentiated while phenelzine alone produced slight hypothermia. In mice, pretreated with reserpine, phenelzine caused analgesia equal to that of morphine, meperidine was inactive, and phenelzine plus either analgesic produced an effect similar to phenelzine alone. All compounds caused hyperthermia in reserpine-treated animals. These results are difficult to interpret, as Gupta and Kulkarni (1966) found that nialamide pretreatment potentiated morphine analgesia in rats, and the usual effect of pretreatment with reserpine was to decrease morphine analgesia (Sigg, 1958; Medakovic, 1964). Jounela and Mattila felt that since meperidine analgesia was

increased by phenelzine and decreased by reserpine, it was more amine dependent than that due to morphine. To explain the interaction they suggested that phenelzine might inhibit the hydrolysis of meperidine, leading to an accumulation of its demethylated metabolite, normeperidine, which had greater toxicity (Miller, 1954). They stated, however, that there was no direct evidence to support this.

Sjoqvist (1965) noted an immediate reaction to meperidine in rats pretreated with pargyline. As the symptoms differed from those caused by large doses of either drug, and the onset was so rapid, he suggested that decelerated metabolism of meperidine was not responsible, and a mechanism involving release of brain amines might be the cause.

Two main lines of reasoning were developed to explain this interaction. It was attributed to (1) inhibition of meperidine metabolism by the antidepressant, leading to elevated levels of either meperidine or normeperidine, which then caused the toxicity, or (2) to an action mediated by alterations in the chemical transmitters of the brain due to the combined influence of both drugs. Both hypotheses were deduced from indirect evidence, gained either by observing the phenomenon in animals, or from clinical experience. More specific investigations into the metabolism of meperidine have helped to clarify the first hypothesis.

The inactivation of meperidine is achieved by two

pathways, as proposed by Plotnikoff et al. in 1952. The drug may be hydrolysed to meperidine acid and ethanol or N-demethylated to normeperidine and formaldehyde. Normeperidine may then be hydrolysed to normeperidinic acid. Meperidinic acid is not demethylated (Burns, 1955), Figure 24.

In the rat, it has been estimated that 16% of a dose of meperidine (Plotnikoff, 1952) and 5 to 10% of a dose of morphine (March and Elliot, 1952) is demethylated in 2 hours.

Meperidine in man is excreted in the urine in roughly the following amounts: 5% unchanged, 5% normeperidine, 12% meperidinic acid, 12% normeperidinic acid. As this accounts for only 34%, other unknown metabolites are probably involved (Burns, 1955). The half life in humans is 3 to 4 hours; in the rat and mouse it is much shorter.

The highest concentrations of demethylating enzyme occur in liver microsomal fractions, although a low activity has been demonstrated in rat brain (Clouet, 1964). N-demethylation of morphine and codeine occurs in a similar manner (Plotnikoff, 1952; Axelrod, 1956). The demethylated derivatives of both morphine and meperidine are less analgesic and more toxic in mice than the parent compounds. When compared to the parent analgesics, normorphine showed a greater difference in analgesia and toxicity than normeperidine (Miller, 1954).

MAO inhibitors have been known for some time to inhibit

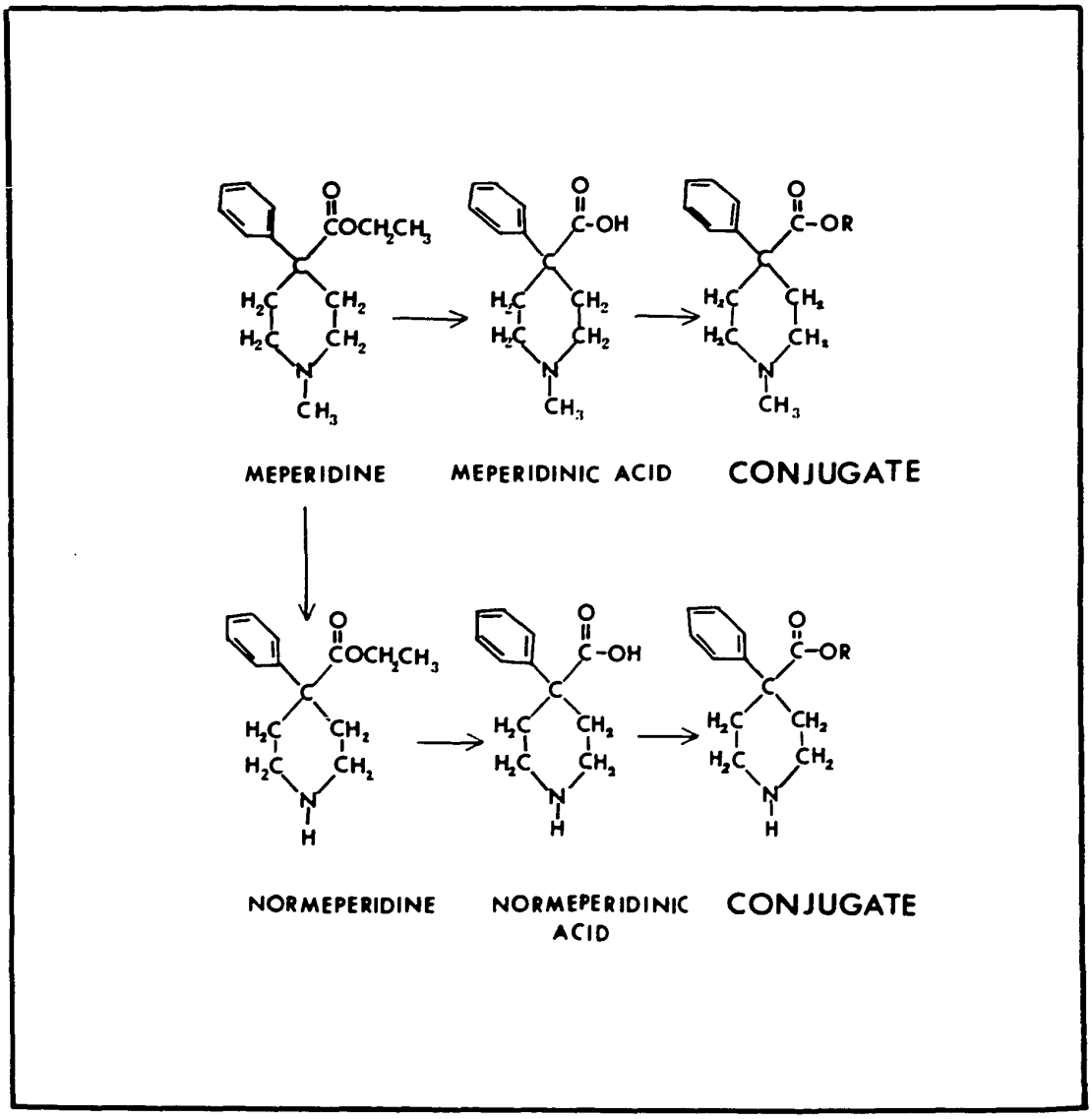


Figure 24: The metabolism of meperidine (Plotnikoff, 1956).
R = unidentified conjugate.

liver microsomal enzyme systems which metabolize drugs (Fouts and Brodie, 1956). It has been suggested that the toxicity of meperidine in patients receiving MAO inhibitors could be explained by an inhibition of hydrolysis leading to increased levels of normeperidine, which is more toxic than the parent compound (Jounela and Mattila, 1968). Clark, in 1967, proved the reverse to be true for in vitro preparations of rat and rabbit liver microsomes, that is, phenelzine competitively inhibited N-demethylation and had no effect on hydrolysis. Normeperidine and meperidinic acid have been given intravenously to man (300 mg and 180 mg respectively) during a study of their metabolism, and no adverse effects were noted from either derivative (Burns, 1955). These metabolites, then, probably do not account for the drug interaction, although this mechanism is often referred to as an explanation in standard texts (Jarvick, M.E., In: The Pharmacological Basis of Therapeutics, 1965; Giarman, N.J., In: In Drills Pharmacology in Medicine, 1965).

The possibility that accumulated meperidine accounts for the toxic interaction has been thoroughly investigated. Phenelzine retarded the in vivo rate of disappearance of meperidine as evidenced by higher meperidine levels in homogenates of whole mice pretreated with the MAO inhibitor, compared to animals receiving the analgesic alone. In the rat, urinary output of meperidine increased, while normeperidine levels decreased after phenelzine pretreatment (Jounela, 1968). Eade and Renton (1970a)

have shown in the mouse that phenelzine increased the rate of recovery of meperidine.

Tranylcypromine was more potent in this respect than phenelzine, but chronic treatment for 7 days produced no greater effect than a single dose of tranylcypromine. Impairment of microsomal enzymes by MAO inhibitors has been shown to occur only in the presence of the drug (Laroche and Brodie, 1960), in contrast to the effect on MAO which persisted for some time after the antidepressant was metabolized. This was confirmed by Eade and Renton (1970a, b), as the extent of inhibition of meperidine metabolism was linearly related to the serum concentration of phenelzine. With respect to toxicity of the combination, phenelzine decreased the LD50 of meperidine, while tranylcypromine did not, indicating no direct connection between inhibition of meperidine metabolism and acute toxicity, as the latter was the more potent inhibitor.

A more detailed <u>in vitro</u> study of the inhibition of meperidine breakdown was carried out using four different MAO inhibitors, d- and l-amphetamine, and SKF-525A, a microsomal enzyme inhibitor (Eade and Renton, 1970b). All compounds tested were found to inhibit meperidine N-demethylase. As shown by Lineweaver-Burke plots, phenelzine and iproniazid, both hydrazines, as well as the amphetamines and SKF-525A produced competitive inhibition, while pargyline and tranylcypromine were non-competitive inhibitors. Pargyline was the only MAO

inhibitor which blocked meperidine esterase, and the inhibition was competitive.

Amphetamine also inhibited meperidine inactivation, but no adverse reactions have been reported for this combination. The authors concluded that while an elevated meperidine level may contribute to the toxicity in man, it is probable that another mechanism is involved.

Burns (1955) has shown that in contrast to rodents, only 10 to 20% of meperidine is metabolized per hour in humans. Partial inhibition of this inactivation would thus elevate blood levels only slightly. Larger doses than those causing reactions in patients treated with MAO inhibitors can be safely administered to man. Furthermore the reaction can occur almost immediately, too soon for impaired metabolism to be involved. Thus it is unlikely that meperidine itself can account for the toxicity. Rogers and Thornton (1969) have also come to this conclusion. In their experiments, pretreatment with tranylcypromine or iproniazid increased the toxicity and central excitation in mice to four narcotic analgesics, morphine, meperidine, phenazocine and pentazocine. Most deaths occurred within 30 minutes, those animals surviving 1 hour usually recovered. Blood levels of pentazocine were the same with or without tranylcypromine pretreatment; thus, the toxicity could not be attributed to higher levels of the analgesic.

B. PHENELZINE

The effects of phenelzine in elevating all three brain amines were predictable from its known action on MAO (Chessin et al., 1959). That dopamine levels rise to a greater extent than noradrenaline may be explained by the pattern of catecholamine synthesis in the brain. Dopamine is not only acted on by MAO and COMT but also is converted to noradrenaline, and this conversion may be blocked by feedback inhibition (Neff and Costa, 1968; Lin et al., 1969a). 5-Hydroxytryptamine also rises to a greater extent than noradrenaline, probably because oxidation by MAO is the main route of metabolism for this amine (Tozer et al., 1966) and its synthesis is not affected by feedback inhibition (Lin et al., 1969a). 5-Hydroxytryptamine has been shown to rise to a greater extent than noradrenaline after iproniazid (Spector et al., 1959), and pheniprazine (Brodie et al., 1959) in the rabbit.

A discrepancy appears when the results of these experiments are compared to the results of McNeill and Riedel (1964), who studied the effects of phenelzine in the same strain of rats and reported that the drug (30 mg/kg, ip) had no effect on brain noradrenaline, while it elevated 5-hydroxytryptamine. As this inhibitor is an effective antidepressant, they proposed that the clinical effects of MAO inhibitors might be due to their effects on brain 5-hydroxytryptamine, and that catecholamine elevation was not an essential factor. In the controversy over which amine might be more responsible for the action of MAO

inhibitors, their work has been quoted (Lapin and Oxenkrug, 1969) to support 5-hydroxytryptamine elevation as the anti-depressant factor. The most likely reason for the discrepancy is the time sequence used by McNeill and Riedel (1964), who measured the amines at 3, 20, 36 and 72 hours after the drug. The level of noradrenaline at 3 hours was 16% above control, although this rise was not significant, and 5-hydroxytryptamine had significantly increased by 75% at this time. In the present experiments, the largest increases in noradrenaline occurred at 6 and 12 hours, whereas 5-hydroxytryptamine rose more rapidly, and the largest increases were seen at 3 and 6 hours. Thus it is likely that an effect on noradrenaline occurred which McNeill and Riedel overlooked by not examining the interval between 3 and 20 hours. Other MAO inhibitors such as pheniprazine (Maynert and Klingman, 1962), nialamide (Gunne, 1963), pargyline (Maickel et al., 1967; Miller et al., 1968) and tranylcypromine (Schildkraut, 1970; Akera and Brody, 1968) have been shown to elevate brain noradrenaline in the rat. Christmas et al. (1970) reported that phenelzine did so as well, although they did not describe the dose or time sequence used. The long time course of elevation of all three amines is considered to be responsible for the delayed onset of the therapeutic effect, which develops slowly after administration.

The decrease in rectal temperature caused by phenelzine agrees with the theory assigning brain noradrenaline and 5-hydroxytryptamine a role in temperature regulation, as an

elevation in either of these amines would be expected to decrease body temperature. The temperature decrease was long lasting (over 6 hours in those animals observed for that length of time), which correlates with the long-lasting effect on both noradrenaline and 5-hydroxytryptamine. Phenelzine (60 mg/kg) has been shown to decrease body temperature in mice at 15 and 60 minutes after administration (Jounela and Mattila, 1968).

C. MEPERIDINE

The effects of meperidine alone on brain amines are less easy to interpret than those of phenelzine. No significant trend occurred with noradrenaline, although in one series of experiments slight, nonsignificant increases were seen (Table 4). Regarding dopamine, a definite trend was apparent. Although none of the values from treated animals were significantly greater than the control value, all of them (7/7) were higher. The probability from the binomial distribution of this occurring by chance alone is 0.007, which makes it most likely that meperidine has some effect on brain dopamine than can be partially compensated for under normal circumstances. A study of individual areas of the brain might reveal a very marked change in a particular area.

With regard to 5-hydroxytryptamine, meperidine seemed to decrease brain levels, although the effect just fails to reach significance, the probability of 6/7 means from treated animals being less than controls is 0.055 (Table 12). That meperidine increased the variability in rectal temperature but produced no

definite response in either direction was consistent with its effect on amines because an increase in dopamine would be expected to lower temperature, while a decrease in 5-hydroxytryptamine might conceivably elevate it. In mice, Jounela and Mettila (1968) observed that 30 mg/kg lowered body temperature at 15 minutes, but had no effect at 1 hour. Lower doses were ineffective.

D. PHENELZINE PLUS MEPERIDINE

The results from experiments in which both drugs were used tend to support the theory that alterations in brain amines are involved in the toxic interaction. Noradrenaline levels rose to a maximum of 103% greater than controls after both drugs. This was higher than the maximum increase seen after twice that dose of phenelzine alone (74%). The effect on dopamine was more striking; the level of this amine increased by 394% in the brain after both drugs, whereas the highest elevation seen after phenelzine alone was 194%.

The effect on 5-hydroxytryptamine was notably different than for the catecholamines, as levels decreased after phenelzine plus meperidine when compared to those animals receiving phenelzine alone. A related study was done by Rogers and Thornton (1969). In one series of experiments they measured MAO activity in brain and liver of mice along with brain levels of 5-hydroxytryptamine, noradrenaline and dopamine, at 2, 4, 8, 16 and 24 hours after the administration of a fixed dose of

tranylcypromine alone. Liver and brain MAO were maximally inhibited at 2 hours; however, levels of brain 5-hydroxytryptamine and dopamine reached a peak at 4 hours, and brain noradrenaline did not do so until 8 hours after the MAO inhibitor. The percent increase of 5-hydroxytryptamine was considerably greater than for the catecholamines. A group of animals received tranylcypromine followed by meperidine at the same time intervals, and the LD50's of the analgesic were determined. Meperidine was most toxic 4 hours after tranylcypromine (Figure 25). When Rogers and Thornton measured the same parameters with increasing doses of tranylcypromine and the time constant at 4 hours, they found linear increases in meperidine toxicity and brain 5-hydroxytryptamine concentrations, whereas MAO inhibition and brain dopamine and noradrenaline elevation reached a plateau at low doses (Figure 26). Thus the toxicity of meperidine after tranylcypromine closely followed the increase in brain 5-hydroxytryptamine caused by the MAO inhibitor. The authors concluded that, since the symptoms of meperidine toxicity after the MAO inhibitor showed greater evidence of central stimulation than did meperidine toxicity alone, and since the maximal toxicity occurred at times when the brain 5-hydroxytryptamine levels were highest after tranylcypromine, the interaction might be related to increased levels of 5-hydroxytryptamine in the brain. They believed a critical level of the monoamine might be necessary before the reaction occurred. They did not examine what happened to these levels when meperidine was given after the MAO

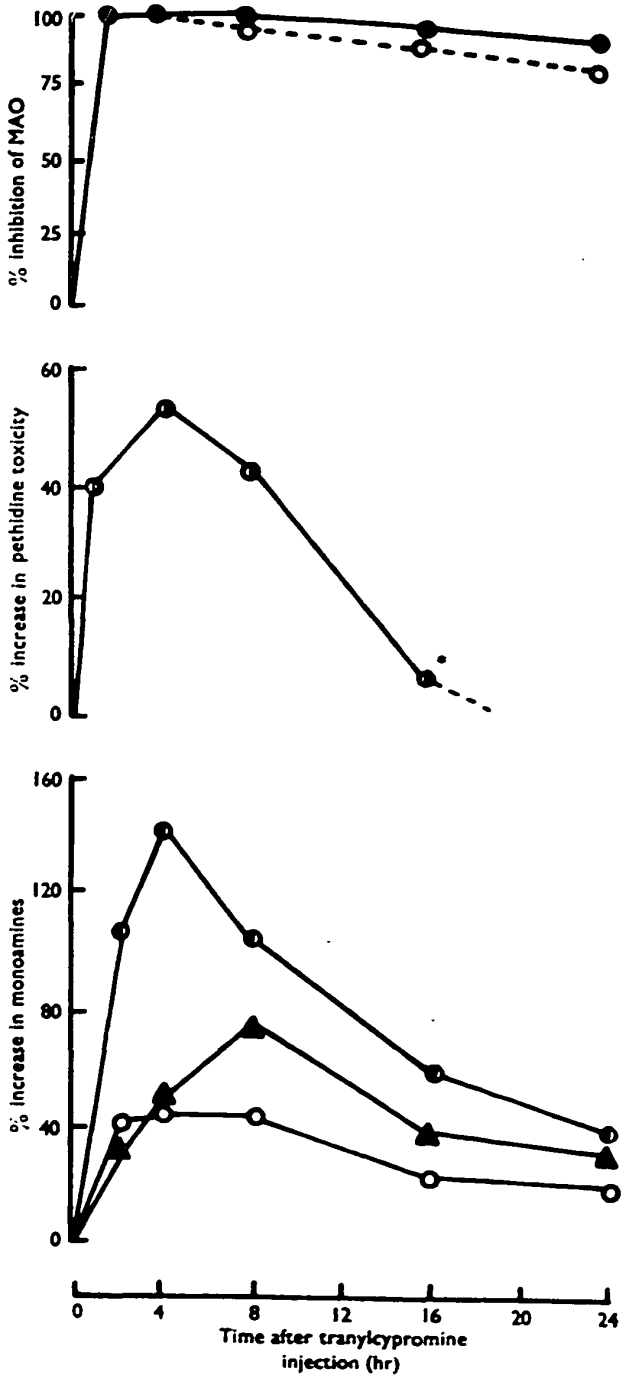


Figure 25: Time course of the effect of tranylcypromine (15 mg/kg intraperitoneally) on brain (●—●) and liver (○--○) monoamine oxidase activity, acute meperidine toxicity (◐—◐) and on the brain concentration of 5-hydroxytryptamine (◐—◐), noradrenaline (▲—▲) and dopamine (○—○) in mice. (From Rogers and Thornton, 1969.)

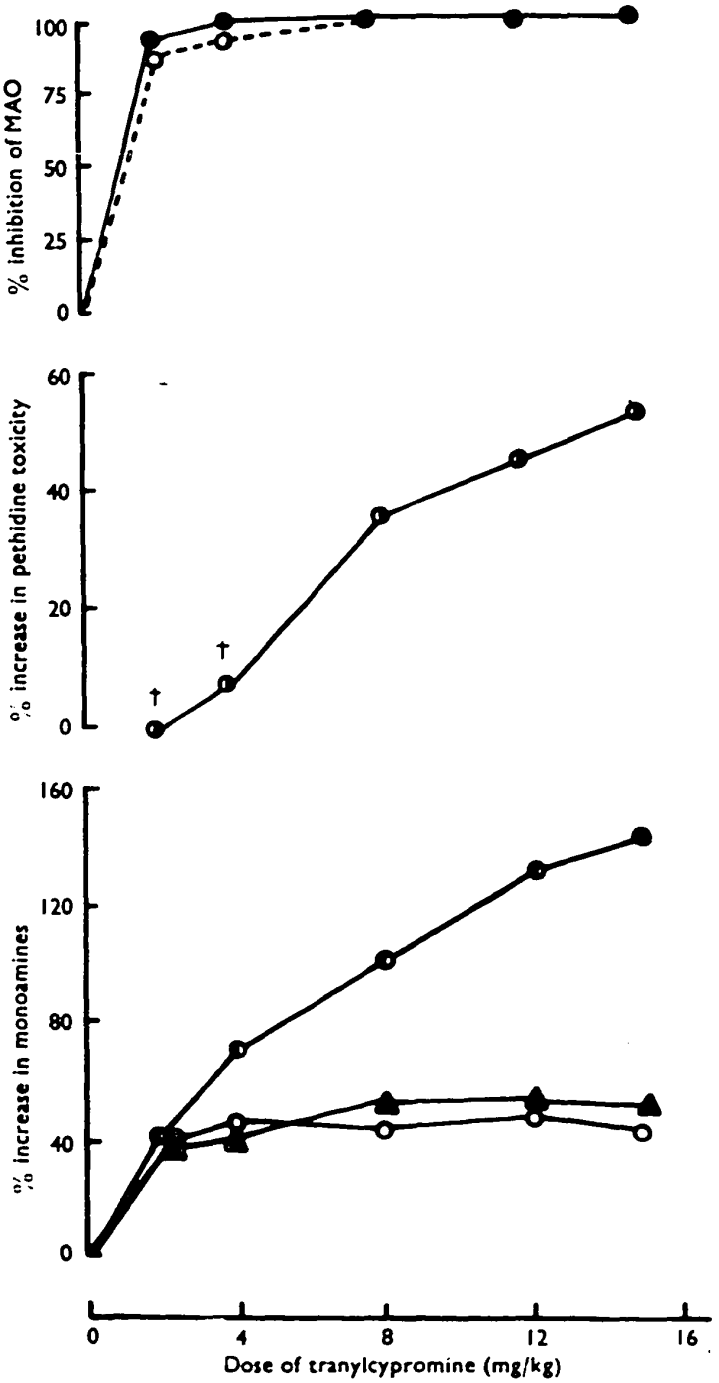


Figure 26: Effect of different doses of tranylcypromine on brain (●—●) and liver (○--○) monoamine oxidase activity, acute meperidine toxicity (◑—◑) and on the brain concentration on 5-hydroxytryptamine (●—●), noradrenaline (▲—▲) and dopamine (○—○) in mice. The animals were killed 4 hours after the intraperitoneal injection of tranylcypromine. (From Rogers and Thornton, 1969.)

inhibitor or what effect meperidine alone had on brain monoamines. If, as Rogers and Thornton proposed, the toxicity after both drugs was related to the elevated 5-hydroxytryptamine, then an explanation relating their proposal to the results of the present study would be that meperidine was capable of releasing 5-hydroxytryptamine, and after MAO inhibition there was more of the amine available to be released. This released amine would react with postsynaptic receptors causing a response, and once the amine was outside of the neuron, other mechanisms (acetylation, conjugation) would be able to metabolize it, thus causing the level in whole brain to decrease. That these mechanisms can dispose of 5-hydroxytryptamine when levels become abnormally high has been demonstrated (Lin et al., 1969b).

There is evidence that re-uptake of both noradrenaline and 5-hydroxytryptamine into central neurons may be blocked by meperidine but not by morphine (Carlsson and Lindqvist, 1969). Although MAO-inhibitor pretreatment has been shown to decrease the LD50 of morphine in laboratory studies (Mustala and Jounela, 1966), there are no documented cases of a clinical interaction occurring with morphine. Perhaps the effect on nerve re-uptake of amines is important in this respect.

The symptoms of the phenelzine-meperidine interaction in rats were very similar to those observed in humans, with an initial hyperactive stage sometimes followed by a coma and death. Although LD50's were not determined in the present study, it was evident that pretreatment with phenelzine increased the

toxicity of meperidine at the time of maximal amine elevation (Section C, Results). The toxicity of the combination does not seem to depend on the concentration of either the MAO inhibitor or meperidine in the body.

It was shown (Eade and Renton, 1970a) that meperidine was more toxic 4 hours after phenelzine (40 mg/kg) than 1 hour after pretreatment, yet serum phenelzine levels were 4.5 times higher at 1 hour than at 4 hours, and the percent meperidine still present in the body was also greater at 1 hour than at 4 hours (65.9% at 1, and 41.2% at 4 hours).

The levels of brain amines, however, would have been higher at 4 hours than at 1 hour, as these rise slowly after MAO inhibition. In this respect, significant increases in analgesic toxicity after tranylcypromine only occurred during the phase of highest elevation of amines (Rogers and Thornton, 1969). If, as is postulated, the interaction requires a certain preexisting high level of brain amines at the time of meperidine administration, the varying severity of the phenomenon might be explained. With the differing doses of various inhibitors administered clinically and the variety of times between the last dose of antidepressant and injection of the analgesic, not all patients would be expected to have exceptionally high amine levels at the time of administration of the second drug. Rogers and Thornton found that low doses of tranylcypromine did not increase meperidine toxicity.

Tranylcypromine (5 mg/kg) had no effect on the LD50 of meperidine when tested by Eade and Renton (1970a). Although this dose was effective in inhibiting microsomal enzymes, it probably did not produce a large change in brain amines as 80 - 85% inhibition of MAO is necessary before the monoamine content of brain increases (Chessin et al., 1959; Pletscher, 1966). The dose of analgesic would also be a factor in the severity of the reaction, as seen in the present experiments. A related clinical study done by Evans-Prosser (1968) using 15 patients who were being treated with various MAO inhibitors showed that doses of 5, 10, 20 and 40 mg of meperidine administered to the same patients 45 minutes apart produced no severe response, only mild discomfort and slight fluctuations in blood pressure in some patients. The doses used in clinical reports of the interaction were never below 100 mg of meperidine given at once (Shee, 1960; Pells-Cocks and Passmore-Rowe, 1962; Taylor, 1962; Denton et al., 1962), and in one case (Papp and Benaim, 1958) 100 mg produced no response, but a second dose of 100 mg 30 minutes later provoked a severe toxic reaction. Thus a critical level of meperidine apparently is necessary for the interaction to occur.

Although the results mentioned above support an involvement of all three amines in the phenelzine-meperidine interaction, a great deal of work would be required to determine the exact mechanism. Separate analyses of individual brain areas could be performed to see if the changes were localized in a specific

area. A simple approach which might be of some use would be to administer reserpine, then phenelzine, then determine the LD50 of meperidine with the various pretreatments. Reserpine would counteract the effect of phenelzine on all three amines, and if no symptoms occurred upon the administration of meperidine, this would further support the role of monoamines in the interaction. α-methyltyrosine could be used to decrease catecholamines selectively, and to study the involvement of 5-hydroxytryptamine. This type of experiment using three drugs in sequence is not uncommon (Takagi et al., 1964; Brodie et al., 1956; Carlsson, 1966), however, it would still leave many questions unanswered. More relevant would be an examination of the turnover rate for each amine. Administration of radioactive precursors could be used to determine the rates of synthesis, and measurement of the metabolites would provide an estimate of the rate of degradation (Lin et al., 1969a; Persson, 1969). It is possible for the absolute level to remain constant while the turnover of amine could increase or decrease considerably. Injection of radioactive amines directly into the brain could be used to estimate the effects of both drugs on re-uptake of released transmitters. Possible in vitro studies could include a fractionation of brain components, and measurement of the effect of both drugs on the uptake of amines by isolated vesicles.

The treatment of this interaction in humans has been mainly supportive. It was suggested by Brownlee and Williams

(1963) that nalorphine might be the agent of choice, as they believed the symptoms were due to a decreased metabolism of meperidine, and a narcotic antagonist might help. London and Milne (1962) recommended acidification of the urine to help increase the excretion of meperidine. The amine hypothesis, although it had not been directly examined, was proposed in 1963 by Nymark and Nielson, who suggested that chlorpromazine might be an appropriate agent in treating the interaction, as it has been shown to have a central antiadrenergic effect, to enhance the formation of O-methylated metabolites of noradrenaline and dopamine after MAO inhibition (Carlsson and Lindqvist, 1963), and also to antagonize some of the effects of 5-hydroxytryptamine in the brain. When chlorpromazine was administered to patients showing this interaction, it was found to be of value (Papp and Benaim, 1958), and has been recommended as the agent of choice for treatment of the hyperactive symptoms (Jacobson, 1965). The results of this study provide some theoretical basis to support the use of chlorpromazine in the treatment of patients undergoing this drug interaction.

V. APPENDIX

APPENDIX

DRUGS

Description

Phenelzine sulphate was obtained from Warner-Chilcott, Lot 8812. The molecular weight of the salt is 234 and the base constitutes 58.2% by weight.

Meperidine (Pethidine hydrochloride) was obtained from May & Baker Ltd., Montreal, Lot 8277. The salt has a molecular weight of 283.8 and the base constitutes 87.7% by weight.

Preparation and administration

All solutions for animal experiments were prepared immediately before use. For each dose to be administered, a different solution was prepared such that a constant volume of 0.1 ml/100 g of body weight was injected. Thus to administer x mg/kg, x/ (percent free base) mg of salt was dissolved per ml of physiological saline.

AMINE STANDARDS

Description

The following compounds were purchased from Calibiochem, Los Angeles, California:

(-) Arterenol Bitartrate Hydrate, B grade, 50.16% base by weight

3-Hydroxytryptamine HCl, A grade, 80.76% base by weight

Serotonin Creatinin H_2SO_4 Complex H_2O, B grade, 43.46% base by weight. These were kept refrigerated at 6°C in dark brown bottles.

Preparation of solutions

Amine stock solutions were prepared monthly. Amounts of noradrenaline and 5-hydroxytryptamine equal to 25 mg free base and an amount of dopamine equal to 50 mg free base were dissolved in 100 ml of 0.01 N HCl; these solutions were stored in the refrigerator. Stock solutions were examined for stability, and retain their amine content for at least 6 weeks.

For each experiment a series of dilutions was made such that all internal standards could be added to the brain homogenate in a constant volume. One ml from each stock solution was added to 22 ml 0.005 N HCl to give a common solution containing 10 µg/ml of noradrenaline, 10 µg/ml 5-hydroxytryptamine, and 20 µg/ml of dopamine. From this, further dilutions were made, yielding 2, 4, 6 and 8 µg/ml of both noradrenaline and 5-hydroxytryptamine, and twice those concentrations of dopamine. These final solutions were used as internal standards, a volume of 0.1 ml being added from a glass micropipette to each tube of brain homogenate. The larger range of concentrations for internal standards of dopamine was chosen as it was expected that endogenous dopamine levels in the brain would be higher than those of the other two amines (Ansell and Beeson, 1968).

REAGENTS

Distilled, deionized water was used for the preparation of all reagents. Unless otherwise indicated, chemicals were

obtained from (1) J. T. Baker Chemical Co., Phillipsburg, N.J., or (2) Fisher Scientific Company, Fair Lawn, N.J. The numbers within parentheses have been used throughout the text to indicate either of these two suppliers. All chemicals and solvents were analysed reagent grade.

Acidified butanol reagent

An aliquot of 1500 ml of 1-butanol (1) was placed in a separating funnel fitted with a teflon stopcock and was washed successively with:

150 ml of 1N NaOH (2)

150 ml of 1N HCl (2)

4 x 150 ml H_2O

The butanol was then saturated with sodium chloride (1). To this was added 1.28 ml of concentrated HCl (36.5 - 38.0%, The McArthur Chemical Co. Ltd., Montreal), and the mixture shaken. Potassium metabisulfite, 1.5 g (1) and 0.15 g ethylenediaminetetraacetate (EDTA), disodium salt (1) were added and the mixture was shaken thoroughly. This reagent was prepared the day before use and stored in the freezer.

Phosphate Buffer, pH 6.5, 0.5 M

To 950 ml of water was added 71.0 g of Na_2HPO_4 (1) and the pH of the solution was adjusted to 6.5 with phosphoric acid (Anachemia Chemicals, Ltd., Montreal). Water was then added to make a total volume of 1 liter.

EDTA reagent, pH 6.5, 0.1 M

Disodium EDTA dihydrate, 3.72 g, was dissolved in 95 ml of 1 M sodium acetate (2) and adjusted to pH 6.5 with 10 N NaOH (2). Water was added to make a final volume of 100 ml.

Iodine reagent, 0.1 N

To 100 ml of pure, absolute ethanol (Gooderham & Worts Ltd.) was added 1.27 g Iodine (1). This was stored in a dark brown bottle.

Alkaline Sulfite

Fifty g of hydrated sodium sulfite (1) was dissolved in 100 ml of water. This was frozen in 1 ml portions in separate tubes. Immediately before use one portion was thawed and 9 ml of 5.0 N NaOH was added and the mixture shaken thoroughly.

Orthophthaldehyde

This chemical was purchased from K & K Laboratories, Inc., Plainview, N.J. Upon receipt it consisted of large dark brown crystals and required recrystallization. The polymerized OPT was dissolved in petroleum ether (1) at 80°C until saturation was achieved. It was then left to cool slowly at room temperature and filtered to collect the crystals. This procedure was then repeated twice and yielded very delicate, light yellow crystals. These were stored in a freezer, (-15°C) in a small bottle wrapped in aluminum foil. The crystals could be kept under these conditions for months without polymerizing.

Acetic Acid, 6 N

To 65 ml of water was added 29.1 ml glacial acetic acid (99.7%, Anachemia Chemicals Ltd., Montreal). Water was added to make 100 ml final volume.

Isooctane

2, 2, 4-Trimethylpentane, GC-Spectrophotometric Quality Solvent (1) was stored in dark brown bottles away from heat.

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