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COMPARISON OF ACTIONS AND INTERACTIONS OF DOPAMINE-BASED ISOQUINOLINES AND THEIR NONCYCLIZED PROGENITORS

by

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Submitted in partial fulfillment
of the requirements for the degree of
Doctor of Philosophy

Faculty of Graduate Studies
The University of Western Ontario
London, Ontario

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ABSTRACT

The formation of aberrant neuroamine metabolites has been postulated in the enhancement of ethanol-induced narcosis by dopaminergic agents. Accordingly, the isoquinoline 3-carboxysalsolinol (3cSAL), the condensate of acetaldehyde with L-DOPA, and salsolinol (SAL), that of acetaldehyde with dopamine, were compared with their neuroamine precursors in alterations of the depressant effect of ethanol (87 mmoles/Kg). The ability of the isoquinolines to affect other behaviours was examined also. Inhibitors of enzymes involved in ethanol and neuroamine metabolism were included in some of the tests.

3cSAL (15, 30, or 60 μmoles/Kg), SAL (460 or 920 μmoles/Kg), L-DOPA (1000 μmoles/Kg) and dopamine 60 μmoles/Kg) increased the duration of ethanol-induced narcosis in mice, but produced no loss of righting reflex when administered alone. The most potent compound was 3cSAL. Pyrazole (0.5 or 1 mmole/Kg) or disulfiram (0.25, 0.5 or 1 mmole/Kg) prolonged ethanol narcosis. 3cSAL (60 μmoles/Kg) prolonged the pyrazole (0.5 mmoles/Kg)-ethanol response. SAL (920 μmoles/Kg) abolished the enhancement in pyrazole-treated animals. L-DOPA (60 or 1000 μmoles/Kg) increased the duration of the disulfiram (0.25 mmoles/Kg)-ethanol response, and salsolinol (920 μmoles/Kg) attenuated the response. Pargyline pretreatment (51, 102 or 510 μmoles/Kg) prolonged the ethanol response, but the amines (60 μmoles/Kg)

exerted no effect on the pargyline (51 mmoles/Kg)-ethanol response. L-DOPA (250 or 1000 μmoles/Kg) prolonged narcosis produced by ethanol with carbidopa (102 μmoles/Kg) or benserazide (1.7 mmoles/Kg). 3cSAL (7.5 or 60 μmoles/Kg) but not SAL (460 μmoles/Kg) enhanced the narcosis response to ethanol and carbidopa, and 3cSAL (60 μmoles/Kg) decreased the response to ethanol and benserazide. The results suggest that formation of isoquinolines in vivo may be involved in the depressant effects of ethanol. L-DOPA and 3cSAL, in the same dose range as employed in the ethanol tests and in the presence and absence of carbidopa produced no change in the duration of hexobarbital (0.39 mmoles/Kg)-induced narcosis in mice.

In the murine tail-clip test for analgesia 3cSAL (2.2 to 880 μmoles/Kg) generated a biphasic dose-response pattern. It was less potent than morphine (11, 16.5 and 22 μmoles/Kg) and more potent than L-DOPA (4.4, 44 and 220 μmoles/Kg). 3cSAL (220 μmoles/Kg) but not L-DOPA (220 μmoles/Kg) increased morphine-induced analgesia (11 μmoles/Kg). Carbidopa pretreatment (102 μmoles/Kg) shifted the 3cSAL pattern of responses towards lower doses, and the isoquinoline enhanced the carbidopa-morphine (11 μmoles/Kg) response. Naloxone administration (3 μmoles/Kg) abolished the analgesia produced by the various treatments. The results suggest that 3cSAL or a metabolite influences central opiate receptor mechanisms.

Spontaneous locomotor activity of mice was increased by a low dose of 3cSAL (38 μmoles/Kg) and decreased by a higher dose (190 μmoles/Kg). Involvement of 3cSAL or a metabolite with dopaminergic motor-

activity centres is implied.

To determine whether 3cSAL can be decarboxylated by L-DOPA-like mechanisms mice were injected with (^{14}C-COOH)-3-carboxysalsolinol (107 or 745 μmoles/Kg) or (1-^{14}C)-DOPA (120 μmoles/Kg), placed in chambers and their expired air bubbled through alkali. The trapped ^{14}C-carbon dioxide declined over 2 hr. For labelled 3cSAL the accumulated radioactivity comprised 0.8% of that administered; for radiolabelled DOPA, 38%. Carbidopa pretreatment (102 μmoles/Kg) suppressed decarboxylation of each compound initially, and later promoted conversion of labelled 3cSAL. In phosphate buffer the labelled isoquinoline (0.86 μmole) and labelled DOPA (1 μmole) decarboxylated nonenzymatically. Addition of guinea-pig kidney homogenate to the incubates increased the levels of decarboxylation. It is concluded that 3cSAL can decarboxylate nonenzymatically and enzymatically more slowly than DOPA. In fluorometric tests 3cSAL (0.25 or 0.5 μmole) produced a dose-dependent increase in the conversion of L-DOPA (1 μmole) to dopamine and carbon dioxide in the presence of kidney homogenate.

The results suggest the involvement of dopamine-based isoquinolines in acute effects of ethanol. Also, the isoquinolines may serve as a link between the actions of ethanol and opiates and may function in dopaminergic mechanisms of motor activity.

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LIST OF ABBREVIATIONS

ADH	Alcohol dehydrogenase
AldDH	Aldehyde dehydrogenase
COMT	Catechol-O-methyl transferase
DBH	Dopamine-beta-hydroxylase
MAO	Monoamine oxidase
NAD	Nicotinamide adenine dinucleotide
P5P	Pyridoxal-5'-phosphate
TBC	Tetrahydro-beta-carboline
THP	Tetrahydropapaveroline
TIQ	Tetrahydroisoquinoline

I INTRODUCTION

Behavioural changes associated with alcohol consumption have been recognized in societies for thousands of years. However, only recently has research into the etiology of alcoholism taken prominent strides. Among the hypotheses presented in this decade is the proposition that in its derangement of neuroamine metabolism ethanol generates tetrahydroisoquinolines (TIQs) capable of contributing to effects observed in alcoholism (Cohen and Collins, 1970; Davis and Walsh, 1970). The authors suggested that such products would be formed by condensation of neuroamines with acetaldehyde, which is produced in ethanol metabolism. One of the TIQs, tetrahydropapveroline, is known to be involved in the early stages of morphine formation in the opium poppy (Kirby, 1967). The suggestion is that TIQs serve as biochemical links between the effects of the two central depressants. By focussing attention on the pharmacology of TIQs the validity of these hypotheses was explored. At the outset of the research little information was available on the acute central effects of TIQs. Moreover, preliminary investigations conducted in other laboratories suggested that isoquinolines related to dopamine may be of particular significance in alcoholism. Therefore, behavioural responses to 3-carboxysalsolinol and salsolinol, acetaldehyde condensates of L-DOPA and dopamine, respectively, were examined in mice. Of special interest were any actions and interactions reminiscent of the acute

effects of ethanol. Alterations of ethanol-induced narcosis by the four agents were studied in the presence and absence of inhibitors of enzymes responsible for the degradation of ethanol or the neuroamines. Pyrazole, disulfiram and pargyline were used to inhibit alcohol dehydrogenase, aldehyde dehydrogenase and monoamine oxidase, respectively. Experiments conducted with the TIQs and central depressants other than ethanol served to delineate the degree of specificity of any TIQ-alcohol interactions observed. The duration of hexobarbital-induced narcosis and the degree of analgesia produced by morphine in the tail-clip test were tested in the presence and absence of 3-carboxysalsolinol and its noncyclized progenitor L-DOPA. Investigations on more subtle changes in behaviour were conducted with the carboxylated TIQ in a spontaneous locomotor activity protocol.

Results from the tests on ethanol-induced narcosis indicated a greater potency for 3-carboxysalsolinol than for salsolinol. It is possible that the carboxylated TIQ achieves better access to the central nervous system than the noncarboxylated congener, a relation suggested by the enhanced central penetration of L-DOPA as compared with dopamine. Accordingly, inhibitors of peripherally- and centrally-located L-aromatic amino-acid decarboxylase were employed to aid in the clarification of the TIQ responses and the degree of central penetration.

In biochemical tests the possibility of the decarboxylation of 3-carboxysalsolinol to salsolinol was examined _in vitro_ and _in_

vivo. Homogenates of guinea-pig kidney served *in vitro* as the source of decarboxylase. Alteration of the decarboxylation of L-DOPA to dopamine by 3-carboxysalsolinol was determined spectrophotofluorometrically. Any associated changes in carbon dioxide production were detected manometrically. Conversion of (1-^{14}C)-DOPA and (^{14}C-COOH)-3-carboxysalsolinol was determined *in vitro* in the presence and absence of kidney homogenate. Finally, the radiolabelled compounds were administered to mice, and expired ^{14}C-carbon dioxide was measured.

II HISTORICAL REVIEW

A. Synthesis of Tetrahydroisoquinolines

1. Chemical Condensation of Neuroamines with Aldehydes

a. The Pictet-Spengler Condensation

In 1911, a report heralded the generation of simple isoquinolines from the condensation of phenylethylamines with carbonyl agents (Pictet and Spengler, 1911). The authors hypothesized that the isoquinoline condensations performed in the chemical laboratory were akin to those resulting in analogous products in plants. Apparently, Schöpf and Bayerle (1934) held the same view, as the conditions of concentration, temperature and acidity employed in their synthesis of the isoquinoline salsolinol were thought to be those present in plants.

Between the time of the initial investigation and the present day, accounts of the chemical and biological synthesis of isoquinolines have sporadically appeared. What probable molecular rearrangements occur during a Pictet-Spengler type of condensation are illustrated in Fig. 1. Generally, the first step involves the linking of a carbonyl group to a phenolic amine that has a free ring position at the carbon atom "ortho" to the ethylamine moiety. This ephemeral intermediate is in the form of a Schiff's base, or, imine. Then, cyclodehydration and the associated closure of the

Figure 1

Formation of neuroamine-based tetrahydroisoquinolines (TIQs) and tetrahydro-beta-carbolines (TBCs)

The condensation of dopamine and serotonin with acetaldehyde yield Schiff's base intermediates, which undergo cyclization to form the TIQ salsolinol (1-methyl-6,7-dihydroxy-1,2,3,4-tetrahydroisoquinoline) and 1-methyl-6-hydroxy-1,2,3,4-tetrahydro-beta-carboline, respectively.

HO
HO
NH_2
CH_3CHO
HO
HO
NH
CH_3
HO
HO
NH
CH_3

Dopamine

A Schiff's base intermediate

Salsolinol

HO
N
NH_2
CH_3CHO
HO
N
NH
CH_3
HO
N
NH
CH_3

Serotonin

A Schiff's base intermediate

1-Methyl-6-hydroxy-1,2,3,4-tetrahydro-β-carboline

ring are encouraged to proceed by virtue of the hydroxyl group located "para" to the site of closure. From the cyclization of phenylethylamines tetrahydroisoquinolines (TIQs) originate; from that of indolethylamines, tetrahydro-beta-carbolines (TBCs), in a similar fashion.

b. Formation of Tetrahydroisoquinolines and Tetrahydro-beta-carbolines

How various isoquinolines and beta-carbolines have been synthesized in the chemical laboratory has been well documented (Whaley and Govindachari, 1951). The techniques that have been used to prepare the aldehyde-based condensates derived from L-DOPA, dopamine, noradrenaline, adrenaline and 5-hydroxytryptamine are briefly outlined in the following paragraphs.

It has been possible to generate particular stereo-specific isomers of the L-DOPA-formaldehyde and L-DOPA-acetaldehyde products, as revealed by Brossi, Forella and Teitel (1972). The "cis" isomer of the Pictet-Spengler condensate of L-DOPA and acetaldehyde, namely, 3-carboxysalsolinol, was employed in many of the tests. It was synthesized according to the method devised by Brossi _et al_. (1972) as described in a later section. Unintentional formation of 3-carboxysalsolinol was observed to occur when tritiated dopa was stored in the presence of an ethanolic preservative (Waldeck, 1973). It appears that acetaldehyde was one of the products created from ethanol exposed to decaying tritiated dopa.

Several isoquinolines with possible biological significance have arisen from the Pictet-Spengler condensation of dopamine with various aldehydes. Buck (1934) described the generation of many hydroxy- and methoxy-substituted TIQs, including norsalsolinol, which is the dopamine-formaldehyde condensate. Salsolinol was made by a simple, direct condensation of dopamine and acetaldehyde, and isosalsolinol was a minor product of the reaction (King, Goodwin and Sandler, 1974). Salsolinol that had been synthesized from homoveratrylamine, a dimethoxylated congener of dopamine, was used in some of the studies presented in this thesis (Hamilton, 1975). The relation of dopamine-based TIQs to their noncyclized progenitors is illustrated in Fig. 2.

More complex isoquinolines have also been generated. For instance, when 3,4-dihydroxyphenylacetaldehyde was produced by the action of monoamine oxidase (MAO) on dopamine, this carbonyl compound united with its parent amine to form tetrahydropapaveroline (THP) (Holtz, Stock and Westermann, 1964a). Pyman (1909) demonstrated the nonenzymatic creation of THP through a process that reduced papaverine.

The chemical syntheses of certain trihydroxyl TIQ congeners of adrenaline and noradrenaline have been reported recently. Mixing acetaldehyde and adrenaline under conditions of optimal temperature, acidity and solvent separation led to the generation of at least two products, one of them being 1,2-dimethyl-4,6,7-trihydroxy-1,2,3,4-tetrahydroisoquinoline (Osswald, Polonia and Polonia, 1975). Sarges

Figure 2

Proposed pathways for the biosynthesis
of L-DOPA-based TIQs

The condensation of L-DOPA or dopamine and acetaldehyde, arising from oxidation of ethanol could produce 3-carboxysalsolinol or salsolinol, respectively. A decarboxylation of the TIQ amino-acid could also generate salsolinol.

An oxidation of dopamine to 3,4-dihydroxyphenylacetaldehyde could be followed by a condensation of the oxidized product with unreacted dopamine to form the benzylisoquinoline tetrahydropapa-veroline.

L-DOPA → (CH_3CHO) → 3-Carboxysalsolinol

CH_3CH_2OH

L-DOPA → ($-CO_2$) → Dopamine

3-Carboxysalsolinol → (? $-CO_2$) → Salsolinol

Dopamine → (CH_3CHO) → Salsolinol

Dopamine → (MAO) → 3,4-Dihydroxyphenylacetaldehyde

Dopamine + 3,4-Dihydroxyphenylacetaldehyde → Tetrahydropapaveroline

3,4-Dihydroxy-phenylacetaldehyde

Tetrahydro-papaveroline

(1974) has provided the details of the synthesis of the noradrenaline-formaldehyde isoquinoline. He produced the compound both from a condensation of the two components and from a sequence of reactions commencing with an alteration of 2,3-dihydroxybenzaldehyde. As the former method yielded a multiplicity of products, the retrieval of the desired substance was a cumbersome process. An earlier report (Collins, 1973) outlined the preparation of not only the noradrenaline-formaldehyde condensate but also the metanephrine- and dopamine-formaldehyde and the noradrenaline- and metanephrine-acetaldehyde isoquinolines. A high degree of purity of the acetaldehyde-based condensates appears to have been difficult to attain.

All of the cyclized products described so far are ones having a catechol nucleus. However, as was mentioned earlier, tetrahydro-beta-carbolines can also be formed, and these compounds arise from indolethylamines. For example, Taborsky and McIsaac (1964) reported the synthesis of the 5-hydroxytryptamine-acetaldehyde compound. A methylated methoxyl beta-carboline has also been prepared, as the product of a Pictet-Spengler condensation (Dajani and Saheb, 1973).

c. Condensation under Pseudophysiological Conditions

Most of the syntheses mentioned in the preceding section were performed by the investigators under rather severe conditions of acidity and temperature. However, many laboratories have reported that rapid production of the condensates is possible at ambient temperature and neutral or near neutral pH. Under these conditions Schöpf and Bayerle (1934) achieved successful condensations of

acetaldehyde with dopamine, producing salsolinol, and with epinine (N-methyldopamine). The Schöpf and Bayerle method was used by Kovacs and Fodor (1951) and by Whaley and Govindachari (1951) to synthesize salsolinol and several other TIQs, all in high yields.

Cohen and Collins (1970) have provided a detailed account of the formation of catecholamine-based isoquinolines under a set of mild conditions. In aqueous buffer L-DOPA, dopamine, noradrenaline and adrenaline reacted with formaldehyde and acetaldehyde to produce their respective isoquinoline congeners. The use of thin layer chromatography helped to identify the major (TIQ) and minor (unknown) products.

It appears that acetaldehyde is capable of condensing with a variety of amines. Walsh (1973) reported the detection of reaction products for catecholamines, tyramine, octopamine, indoleamines, the methoxylated derivatives of many of these substances and for the amino acids from which the biogenic amines arise. How rapidly the amine-acetaldehyde reaction can occur has been examined for some of the neuroamines. At pH 7.4 and 37 C the second order rate constants for acetaldehyde condensations in weak phosphate buffer were determined. For millimolar concentrations of dopamine, L-DOPA and noradrenaline the rate constants were found to be 15.3, 6.1 and 1.9, respectively (Robbins, 1968b). Neither tryptamine nor 5-hydroxytryptamine were observed to react with acetaldehyde under these conditions, even though the test was capable of detecting less than 1% conversion (Robbins, 1968a). However, McIsaac (1961) demonstrated

the formation of a beta-carboline from the reaction of 5-methoxy-tryptamine with acetaldehyde at pH 7 and 37 C.

Under pseudophysiological conditions it has been shown that L-DOPA, dopamine and noradrenaline and other biogenic amines can form TIQs when reacted with the aldehyde pyridoxal-5'-phosphate (Schott and Clark, 1952). In fact, the authors hypothesized that such a formation may be involved in the mechanism by which dopa decarboxylase is inhibited in the presence of L-DOPA and pyridoxine.

2. Isoquinoline Biosynthesis

a. Botanical Formation

Isoquinoline alkaloids are found in abundance in the plant kingdom. But, as widespread as their occurrence is perceived to be, few isoquinolines have as yet been subjected to detailed pharmacological investigation. For reviews of the pharmacognosy of isoquinolines the reader is referred to works by Shamma (1972) and Hirst, Hamilton and Marshall (1977).

Members of the Cactaceae family of plants harbour several simple TIQs. For instance, gigantine, which is a dimethoxylated congener of adrenaline, is produced in the suaharo cactus; certain trioxy TIQs, in the peyote cactus. Methoxylated compounds related to salsolinol, namely, salsoline and salsolidine, are found in Chenopodiaceae species. From Mucuna deeringiana (velvet bean) the isoquinoline 3-carboxysalsolinol has only very recently been

extracted (Daxenbichler et al., 1972). How extensive is its presence in other species of Leguminosae has not been ascertained.

Benzylisoquinoline alkaloids are elaborated by Papaveraceae. It is especially important to note that in *Papaver somniferum* (opium poppy) the benzylisoquinoline tetrahydropapaveroline is formed as an intermediate along the synthetic pathway that converts tyrosine to morphine (Kirby, 1967). The current status of THP as a pharmacological agent will be detailed in subsequent sections. One of the few benzylisoquinolines that have been examined for drug action is the tetramethoxylated compound papaverine, known to relax smooth muscle (Nickerson, 1975).

Alkaloids with a closed ring indolethylamine nucleus in their structure are also produced in a variety of plants. Examples of such compounds are the harmala and yohimbine alkaloids and reserpine, a chemical congener of yohimbine.

b. Formation in Mammalian Systems *In Vitro*

Isoquinolines and beta-carbolines have been generated in tissue preparations obtained from several different mammalian species. By adding the appropriate precursors and cofactors to the chosen *in vitro* systems, researchers have encouraged and subsequently confirmed the formation of neuroamine-derived alkaloids.

Histochemical production of monoamine-formaldehyde condensates was the basis of the fluorescence method devised by Falck and Hillarp for visualizing neuroamines. Exposing dried, proteinaceous monoamine-

containing material to formaldehyde vapour promotes ring closure in the amine and, ultimately, formation of fluorescent tautomers. Requisite molecular intermediates in catecholamine visualization are tetrahydroisoquinolines and their corresponding dihydroisoquinolines. In tryptamine visualization, tetrahydro- and corresponding dihydro-beta-carbolines are involved. This sensitive and specific histochemical technique has been described at length by Corrodi and Jonsson (1967).

Experiments conducted by Holtz et al. (1964a) and most recently by Weiner (1978) demonstrated that THP was formed in rat liver mitochondrial preparations incubated with dopamine. Inhibition by pargyline of monoamine oxidase (MAO) in the incubates prevented THP formation from dopamine and the deaminated aldehyde of dopamine, as determined in part by thin-layer chromatography (Holtz *et al.*, 1964a). Furthermore, when epinine was used as a substrate the authors did not detect formation of a THP congener, for the necessary aldehyde intermediate could not be formed from epinine.

Other authors have also described the biosynthesis of THP *in vitro*. Halushka and Hoffmann (1968) demonstrated THP formation in a guinea-pig liver mitochondrial preparation incubated with dopamine. Davis, Walsh and Yamanaka (1970) illustrated that homogenates of rat liver or brainstem incubated with dopamine yielded significant quantities of the isoquinoline. In the presence of ethanol (100 mM) the amount of THP formed in the liver homogenate was decreased, whereas in the brainstem homogenate it was increased. Acetaldehyde

(4 mM) served to decrease THP generation, and to increase that of salsolinol, in the liver homogenate. The addition of acetaldehyde (2 mM) to the brainstem preparation potentiated THP formation. When exogenous NAD was employed in these tests, the aldehyde-oxidizing capacities of the tissues became apparent, and the isoquinoline fraction, the oxidation product and the reduction product fractions were correspondingly altered.

Evidence of THP synthesis in rat brainstem homogenates has more recently been provided by Turner et al. (1974). Incubation with 50 μM dopamine resulted in the conversion (less than 1%) of the added dopamine to the benzylisoquinoline, as determined by mass fragmentography. In a concentration of 5 mM either dopamine or L-DOPA was converted in part to THP. Preincubation with pargyline prevented the dopamine-induced formation of the isoquinoline.

As mentioned previously, the work of Davis et al. demonstrated the formation of salsolinol, in rat liver and brainstem homogenates (Davis et al., 1970; Yamanaka, Walsh and Davis, 1970). Ethanol (100 mM) and acetaldehyde (4 mM) increased the amount of salsolinol formed in these preparations. In fact, the enhanced synthesis observed with acetaldehyde (0.5 to 4 mM) appeared to occur in a dose-related manner in brainstem incubates.

According to Alivisatos et al. (1973), the brainstem homogenate formation of THP and salsolinol was inhibited by various agents. Ascorbate and glutathione seemed to affect the Schiff's base intermediate in such a way that the amine ring would not close. Of

the agents used, the most effective inhibitor was cysteine. The authors suggested that cysteine blocked isoquinoline synthesis by promoting the creation of a thiazolidine congener.

Vandenheuvel et al. (1975) identified 6,7-dihydroxytetrahydroisoquinoline (norsalsolinol) in a rat brain homogenate incubated with dopamine and methyltetrahydro-(5-^{14}C)folic acid, which acted as a carbon donor. Gas-liquid chromatography used in conjunction with gas-liquid radiochromatography showed that the product of the reaction was norsalsolinol rather than epinine. It is possible that the folate substance was converted enzymatically to formaldehyde prior to condensation with dopamine (Rosengarten, Meller and Friedhoff, 1975).

One of the first reports implicating isoquinolines in alcoholism was the adrenal perfusion study by Cohen and Collins (1970). Using bovine adrenals as a source of catecholamines, they performed perfusions with formaldehyde and acetaldehyde (100 µg/ml). Then, tissue homogenization was conducted, followed by thin layer chromatography. The major reaction products appeared to be the TIQ condensates of the aldehydes with noradrenaline and adrenaline. Tests with ^{14}C-acetaldehyde (1 µg/ml), an acetaldehyde concentration possible in nonalcoholic subjects after ethanol ingestion (Truitt and Walsh, 1970), again indicated noradrenaline- and adrenaline-based TIQs (Cohen, 1971). Other experiments carried out in the same laboratory revealed that the isoquinolines were bound in the same chromaffin granules that contained catecholamines, and that the

isoquinolines and catecholamines were in the same relative proportions both in the gland and in the granules (Greenberg and Cohen, 1972). Once the isoquinolines had been formed in adrenal medullary tissue, they and the catecholamines could be secreted upon perfusion with carbachol or acetylcholine, the secretion being prevented by calcium ion depletion or tetracaine pretreatment (Greenberg and Cohen, 1973). By altering the concentrations of calcium ions and acetaldehyde, Rahwan, O'Neill and Miller (1974) separated the adrenal secretion of isoquinolines from the release of the catecholamines.

Davis _et al_. (1974) reported the formation of a dihydroxylated congener of THP from noradrenaline with the noradrenaline-derived aldehyde. In rat brainstem and liver homogenates the addition of barbiturates or nicotinamide cofactors altered the proportions of the oxidative pathway products, reductive pathway products and the isoquinoline.

Beta-carbolines have been produced in homogenates of rat brain and rabbit lung upon the addition of tryptamines and 5-methyltetrahydrofolic acid to the incubates (Hsu and Mandell, 1975; Rosengarten _et al_., 1975). The authors noted that the condensations necessitated conversion of the folate compound to formaldehyde before the appropriate Schiff's base rearrangements were possible.

The combination of L-DOPA and pyridoxal-5'-phosphate (codecarboxylase) in the presence of a guinea-pig kidney homogenate

resulted in the apparent generation of TIQs (Schott and Clark, 1952). At what time during the incubation the codecarboxylase was added in relation to the time of L-DOPA addition seemed to alter the extent of inhibition of the decarboxylase.

c. Formation in Mammalian Systems *In Vivo*

Any postulated role for isoquinolines or beta-carbolines in alcoholism requires that they be found *in vivo*. Yet, much of the research into this aspect of their character is rudimentary, in many ways hampered by current technological limits.

The groundwork of the whole animal studies was laid by Cohen and Barrett (1969). They found that the administration of methanol to rats resulted in TIQ formation in adrenal tissue, as determined by fluorescence microscopy. Tests repeated with ^{14}C-methanol confirmed by thin layer chromatographic radioassay that adrenaline- and noradrenaline-formaldehyde TIQs were indeed generated (Collins and Cohen, 1970). In 1973, Sandler and coworkers demonstrated for the first time that TIQs were formed in humans after L-DOPA and ethanol ingestion (Sandler *et al.*, 1973). Gas chromatographic and mass fragmentographic evidence was presented as the basis of their findings.

THP formation *in vivo* has been detected in guinea pigs, rats and humans. Halushka and Hoffmann (1968) reported that the isoquinoline was present in guinea pig liver, but no other tissue, at the time of the peak depressor effect of intravenously injected

^{14}C-dopamine (250 μg/Kg). Rats were found to contain THP in brain tissue after an 8-day treatment with L-DOPA (4 mg/ml) and the decarboxylase inhibitor benserazide (1 mg/ml) in the presence and absence of 10% (w/v) ethanol , all of which were delivered in the drinking water (Turner et al., 1974). The amount of the isoquinoline produced in the absence of ethanol was estimated to be approximately 8 ng/g tissue. In the presence of ethanol, THP amounted to 25 ng/g tissue. Both THP and salsolinol were discovered in the urine of Parkinsonian patients who were on L-DOPA therapy (3 to 4 g/day) and had ingested a solution containing 75 ml absolute alcohol (Sandler et al., 1973). Excretion of THP ranged between approximately 30 and 480 μg/12-hr period; salsolinol, approximately 600 and 1850 μg/12-hr period. Recently, it has been observed that two derivatives of THP are also formed in Parkinsonian patients treated with L-DOPA or with L-DOPA and carbidopa, a peripheral decarboxylase inhibitor. The carboxylic acid congener of THP as well as the O-methyl ether of the acid have been detected by mass fragmentography. The former congener has been found in amounts ranging between 0.22 and 1.7 μg/day (Coscia et al., 1977). Comparable findings were made in rats undergoing treatment regimens similar to those employed in humans.

Salsolinol generation in subjects exposed to ethanol has been under study in several laboratories. Collins and Bigdeli (1975) disclosed that when pyrogallol (250 mg/Kg), an inhibitor of catechol-O-methyltransferase (COMT), was administered to rats prior to acute ethanol treatment, approximately 17 ng salsolinol /g tissue were detected in brain by an electron capture/gas chromatography method

(Bigdeli and Collins, 1975). The inclusion of pretreatment with the MAO inhibitor pargyline into the protocol resulted in approximately 118 ng salsolinol/g tissue. The omission of pyrogallol from the test resulted in no apparent salsolinol formation. Furthermore. O'Neill and Rahwan (1975) reported that chronic ethanol administration to mice in the absence of prior pharmacologic manipulation yielded no detectable salsolinol in brain tissue, as analyzed by electron capture/gas chromatography. The lower limit of the assay sensitivity in their technique was 8 ng of salsolinol/g tissue. Sandler had shown earlier that salsolinol was generated in humans after alcohol consumption only if L-DOPA had also been ingested. A more recent publication has announced the presence of salsolinol and norsalsolinol in the urine of humans during either alcohol detoxification or L-DOPA therapy (Nijm et al., 1977). The urinary levels of the TIQs were estimated to be less than 60 ng/ml, as calculated from two different chromatographic techniques.

The conversion of salsolinol to an O-methylated congener appears possible by the action of COMT (Collins, Cashaw and Davis, 1973). Accordingly, the search for 6- or 7-O-methylated salsolinol has been undertaken recently (Hamilton, Blum and Hirst, 1978). The exposure of mice to ethanol vapour for 10 days resulted in the formation of an O-methylated salsolinol in the dopamine-rich corpora striata. By electron capture determination the amount of the isoquinoline formed was 5 to 8 ng/g striatal tissue.

Beta-carboline formation *in vivo* was reported by McIsaac in 1961. Rats were administered ^{14}C-5-methoxytryptamine, the MAO inhibitor iproniazid, ethanol or acetaldehyde, and disulfiram. Subsequent analysis by paper chromatography indicated that 1-methyl-6-methoxytetrahydro-beta-carboline was formed and represented an approximately 0.5% conversion of the administered radioactive agent (McIsaac, 1961).

B. Pharmacology of Tetrahydroisoquinolines

1. Introduction

Isoquinolines have demonstrated the ability to alter many neural characteristics at peripheral and central sites. Descriptions of their observed effects and proposals of their involvement in neural disorders are presented in the following sections.

2. Neurotransmitter Uptake

The interference of TIQs with the movement of neuroamines into nerve tissue has been reported by several suthors. Heikkila, Cohen and Dembiec (1971) demonstrated that salsolinol possessed the ability to inhibit dopamine and noradrenaline accumulation in rat brain synaptosomes. Further work in the same laboratory indicated that norsalsolinol and the stereoisomer S(-)-salsolinol were more potent than R(+)-salsolinol, R(+)-THP or S(-)-THP as inhibitors of ^{3}H-dopamine uptake into rat brain slices (Cohen *et al*., 1974). Recently, Alpers *et al*. (1975) confirmed the synaptosomal findings with the simple TIQs and reported also similar results for the more

complex tetrahydroprotoberberine alkaloids.

In other synaptosomal studies salsolinol and dimethoxysalsolinol were found to be weak inhibitors of serotonin uptake (Tuomisto and Tuomisto, 1973), as was norsalsolinol (Heikkila and Cohen, 1974). It was demonstrated in a recent report that a methoxylated tetrahydro-beta-carboline was also capable of inhibiting serotonin uptake (Buckholtz and Boggan, 1976).

3. Isoquinoline Uptake and Storage

Cohen and his associates have presented evidence for the neuronal uptake of TIQs. The condensates formed from the reaction between formaldehyde and dopamine or noradrenaline were taken up by rat brain homogenates (Heikkila *et al.*, 1971). Mouse heart sympathetic nerve terminals and adrenals, and rat submaxillary glands, irides and adrenals accumulated norsalsolinol following intravenous injection of the isoquinoline (Locke, Cohen and Dembiec, 1973). Desmethylimipramine, an inhibitor of neuronal catecholamine transport, and 6-hydroxydopamine, a denervating agent, inhibited the norsalsolinol uptake into the sympathetically-innervated tissues.

Through the use of the Falck-Hillarp fluorescence microscopy technique (Corrodi and Jonsson, 1967), Cohen demonstrated TIQ storage in rat and mouse iris *in vitro* and *in vivo* (Cohen, Mytilineou and Barrett, 1972; Mytilineou, Cohen and Barrett, 1974). It appears that the TIQs were concentrated at the catecholamine-binding granules in the iris and in the sympathetic glands studied (Greenberg and Cohen, 1972; Tennyson *et al.*, 1973; Schneider, 1974).

4. Catecholamine Release and Depletion

The release, or displacement, of catecholamines by TIQs has been observed in rat brain synaptosomal preparations. Salsolinol, THP and a tetrahydroprotoberberine were found to be effective releasing agents (Heikkila *et al.*, 1971; Alpers *et al.*, 1975). The hypothermia produced in rats by the intraventricular injection of norsalsolinol was thought to have been a consequence of catecholamine release (Brezenoff and Cohen, 1973). In another test performed *in vivo* the administration of 2- and 4-substituted congeners of norsalsolinol led to noradrenaline depletion from mouse hearts (Smissman *et al.*, 1976).

Collins and coworkers recently reported the selective depletion of catecholamines from rat brain regions. Given intraperitoneally, salsolinol and the noradrenaline-acetaldehyde condensate decreased the dopamine level in the brain, but the noradrenaline level was unchanged. The striatal content of serotonin was doubled by treatment with 3-carboxysalsolinol, whereas that of dopamine was slightly decreased. The administration of 3-carboxysalsolinol after pretreatment with a peripheral decarboxylase inhibitor profoundly diminished striatal dopamine amounts. Yet, the hypothalamic noradrenaline, dopamine and serotonin amounts were unaltered by either of these treatments. From the chromatographic analysis it appears that the TIQs were taken up preferentially into striata (Collins, Hannigan and Weiner, 1978; Hannigan and Collins, 1978).

5. Isoquinoline Release

The formation of TIQs _in situ_ during aldehyde perfusion of adrenal glands was noted in a previous section. In the presence of cholinergic stimulation, catecholamines and TIQs could subsequently be released from the glands (Greenberg and Cohen, 1973). A decrease in the concentration of divalent calcium ions in the medium resulted in catecholamine release from the adrenals, whereas the TIQs were retained (Rahwan _et al._, 1974). In the rat iris _in vivo_ norsalsolinol accumulation was depleted by preganglionic stimulation of the superior cervical ganglion, and coincident sympathetic smooth muscle responses of the eye were also observed (Mytilineou _et al._, 1974). The authors noted the likelihood of a neuronal leakage of TIQs in addition to the apparently exocytotic means for TIQ release.

6. Actions of Isoquinolines at Neurotransmitter Receptors

Many investigators have attempted to delineate the actions of TIQs at catecholaminergic and serotonergic receptors. During the past few decades isoquinolines have been observed to affect both central and peripheral neuronal systems.

Effects of TIQs on dopaminergic models are recently reported phenomena. Norsalsolinol stimulated rat striatal adenylate cyclase in a manner akin to that of dopamine, while salsolinol and THP produced no such stimulation (Miller _et al._, 1974). Furthermore, S(-)-salsolinol and both optical isomers of THP were observed to inhibit dopamine-induced adenylate cyclase stimulation (Sheppard and Burghardt, 1974). In a similar assay, which employed epinine

as the agonist, it was confirmed that the THP conformers were more potent adenylate cyclase inhibitors than salsolinol (Sheppard, Burghardt and Teitel, 1976). At the dopamine receptors in a mollusc intestinal preparation stimulated electrically, S(-)-salsolinol behaved as a weak agonist, whereas S(-)-THP antagonized dopamine and S(-)-salsolinol in their actions (Dougan, Wade and Mearrick, 1975).

Isoquinoline alkaloids have displayed agonism or antagonism in several alpha-adrenergic receptor preparations. The earliest findings emanated from the laboratory of Fassett and Hjort (1938) who showed that norsalsolinol and other dihydroxylated isoquinolines increased carotid arterial blood pressure and decreased the heart rate in dogs and cats. In accompanying tests norsalsolinol and salsolinol produced exophthalmos and a pilomotor effect in mice, which are signs of sympathetic stimulation (Hjort, De Beer and Fasset, 1938). Iris contraction and exophthalmos were reported in rats for the accumulated norsalsolinol released by preganglionic stimulation (Mytilineou et al., 1974). Prior treatment with this dopamine-formaldehyde isoquinoline in the rat hypogastric nerve-vas deferens preparation transiently increased the second phase contraction but abolished the initial twitch response (Baird-Lambert and Cohen, 1975). The noradrenaline-formaldehyde condensate, 4,6,7-trihydroxy-tetrahydroisoquinoline, was ineffective in altering either phase of the contraction.

The cardiovascular responses arising from alpha-receptor activation have been examined in greater detail. In pithed rats in the presence of propranolol, the increase in arterial blood pressure observed for norsalsolinol was reduced following sympathectomy or cocaine pretreatment (Simpson, 1975). Dose-dependent elevations in carotid blood pressure as well as dose-dependent contractions of nictitating membrane were observed in dogs upon the intracarotid injection of the adrenaline-acetaldehyde TIQ. The addition of the alpha-receptor blocker phentolamine produced a reduction in the effects (Osswald et al., 1975). These authors noted also that the isoquinoline-induced contractions of isolated venous strips were blocked by pretreatment with phentolamine. When nonsubstituted tetrahydroisoquinoline was administered to dogs, the rise in blood pressure it produced was blocked by pretreatment with the alpha-receptor antagonist phenoxybenzamine (Toth, Fassina and Soncen, 1967).

Some TIQs have themselves demonstrated the ability to block sympathetic alpha receptors. In research on the rat hypogastric nerve-vas deferens preparation, Baird-Lambert and Cohen (1975) performed a prewash with S(-)-salsolinol, which greatly attenuated the twitch and the second phase contraction elicited by electrical stimulation. Racemic 1-benzyl-norsalsolinol demonstrated competitive antagonism of the noradrenaline-induced contraction of guinea-pig aortic strips (Lee et al., 1974), as did salsolinol (Hamilton and Hirst, 1976).

Examination of TIQs in sympathetic beta-receptor systems has yielded much valuable information. Studies performed *in vitro* and *in vivo* have indicated agonistic activity for many isoquinolines, particularly THP.

In isolated guinea-pig atria and Langendorff heart preparations THP has demonstrated positive inotropic effects (Santi *et al.*, 1964, 1967; Feller, Venkatraman and Miller, 1975). Weaker stimulation was apparent for unsubstituted tetrahydroisoquinoline (Toth *et al.*, 1967), S(-)-salsolinol (Feller *et al.*, 1975) and the adrenaline-acetaldehyde product (Osswald *et al.*, 1975). The beta-receptor blocker propranolol was observed to antagonize the cardiovascular stimulation effectively (Santi *et al.*, 1967; Toth *et al.*, 1967; Osswald *et al.*, 1975).

Pithed rats pretreated with phenoxybenzamine showed an increase in heart rate upon norsalsolinol administration (Simpson, 1975). In cats and dogs THP likewise demonstrated a chronotropic effect (Laidlaw, 1910; Holtz *et al.*, 1964; Santi *et al.*, 1964) as well as a lowering of blood pressure (Laidlaw, 1910; Holtz, Stock and Westermann, 1963). When beta-receptor blockers were used, the THP-induced cardiovascular effects were prevented (Holtz *et al.*, 1963, 1964b; Santi *et al.*, 1967). Similar findings have appeared in clinical tests. An increase in heart rate was observed in Parkinsonian patients treated with THP, and the response was greatly attenuated by the prior administration of the beta-receptor antagonist practolol (Dordain, Gouget and Simon, 1974).

Sympathetically-innervated airway smooth muscle has also been used to demonstrate TIQ actions. A trimethoxylated benzyl-TIQ was found to be more active than isoproterenol as a dilator of isolated guinea-pig tracheal muscle (Iwasawa and Kiyomoto, 1967; Feller et al., 1975). On the same preparation the R(+)- and S(-)-conformers of THP also effectively relaxed the muscle strips (Feller et al., 1975). However, the salsolinol isomers or racemate were relatively inactive (Feller et al., 1975; Hamilton and Hirst, 1976). Beta-receptor blockade shifted the THP dose-response curve in a manner typical of competitive antagonism (Iwasawa and Kiyomoto, 1967). Tests performed in vivo have further supported these findings. THP given intravenously to cats demonstrated broncholytic activity (Laidlaw, 1910) that was prevented by beta-receptor blockade (Holtz et al., 1964).

The lipolytic effects observed with several TIQs has exemplified yet another beta-sympathomimetic response. THP has been shown to mobilize free fatty acids from rat adipose tissue preparations (Holtz et al., 1964; Santi et al., 1964). In a study using isolated mouse cells, the S(-)-isomer of THP was found to be the only active conformer (Cohen et al., 1974). Other stereoisomeric tests demonstrated that the S(-)-isomer of 1-benzyl-norsalsolinol was a more effective lipolytic agent than S(-)-THP or S(-)-salsolinol in rat adipose tissue (Lee et al., 1974). Furthermore, the authors showed that both isomeric forms of THP and salsolinol were able to stimulate glycerol release. They indicated that salsolinol was much weaker than noradrenaline with respect to lipolytic activity and agreed with Holtz et al. (1964b) that THP was also less potent than

noradrenaline. In contrast with this latter finding, another report stated that THP and noradrenaline were equipotent in mobilizing free fatty acids in a rat adipose incubate (Gaion *et al.*, 1976).

Isoquinolines related to THP displayed lipolytic activity in rat epididymal fat preparations. A trimethoxy-benzyl derivative of norsalsolinol appeared to be even more potent than isoproterenol or noradrenaline in glycerol release (Feller, Shonk and Miller, 1970; Shonk, Miller and Feller, 1971). For all of the agents tested, the S(-)-isomer in each case was the more active of the two possible conformers.

The administration of isoquinolines *in vivo* resulted in an increase in plasma free fatty in rats (Santi *et al.*, 1964) and in dogs (Toth *et al.*, 1967). Beta-receptor blockade prevented the lipolytic effects seen both *in vivo* (Toth *et al.*, 1967) and *in vitro* (Holtz *et al.*, 1964b; Feller *et al.*, 1970).

In the lipolytic studies performed by Gaion *et al.* (1976), THP demonstrated the ability to increase cyclic AMP levels. The mechanism by which the increase occurred was not clarified. But, it has been well documented that both optical isomeric forms of THP displayed agonist activity at the beta-type adenylate cyclase of the rat erythrocyte (Sheppard and Burghardt, 1974; Sheppard *et al.*, 1976, 1977).

Few investigations have examined the possible effects of TIQs on any other isolated tissue preparation. Santi et al. (1967) did demonstrate that THP inhibited guinea-pig and rat ileal contractions produced by acetylcholine, histamine and barium chloride, and that the spasmolytic effect decreased as the dose of THP was increased. Recently salsolinol was found to competitively antagonize the contractions induced by serotonin on isolated rat fundic strips and estrogen-dominated rat uterine strips (Hamilton and Hirst, 1976). The authors subsequently showed that salsolinol competitively inhibited oxytocin-induced contractions of the uterine muscle preparation and noncompetitively inhibited vasopressin-induced contractions of guinea-pig sacculus (Hamilton and Hirst, 1977). In early research salsolinol was observed to increase the tone of rabbit and guinea pig uterine strips (Hjort et al., 1942).

7. Behavioural Responses to TIQs

Signs of centrally-mediated effects of TIQs have been observed sporadically over the past few decades. By administering the agents either peripherally or centrally into discrete brain regions investigators have more clearly delineated the role of isoquinolines in behaviour.

Simon et al. (1971) disclosed that THP administration to mice and rats caused them to be akinetic and hyperreactive to touch. Hyperthermia was apparent in each species when low doses were given; hypothermia, when higher doses. However, in contrast with the response to certain other TIQs, the delivery of THP directly into

the rat nucleus accumbens produced no change in spontaneous locomotor activity (Costall, Naylor and Pinder, 1976).

Norsalsolinol demonstrated a variety of behavioural effects. Upon bilateral intrastriatal injection of norsalsolinol, unsubstituted norsalsolinol or 3-methyl-norsalsolinol slight dyskinesis was expressed as hyperactivity or abnormal head movements (Costall, Naylor and Pinder, 1975). N-methylation of some of the isoquinolines increased the activity. Propranolol and the dopamine-receptor blocker haloperidol successfully inhibited the hyperactivity generated by 3-methyl-norsalsolinol (Costall _et al._, 1976). In the same report it was stated that the pronounced stimulation apparent with a 3-methyl-methylenedioxyl compound was abolished only by haloperidol.

In the initial toxicological studies conducted by Hjort _et al._ (1938) high doses of norsalsolinol and related N-methyl compounds were observed to produce in mice central depression with terminal convulsive movements. Methoxylated agents caused depression usually unaccompanied by pronounced tremors or spasms. In frogs the central depression included an obvious loss of muscle tone. In mice, intracerebral injection of salsolinol produced an open field immobilization response (Blum _et al._, 1978). Intraventricular injection of norsalsolinol into rats led to a sudden, dose-related fall in body temperature, a response inhibited by 6-hydroxydopamine pretreatment (Brezenoff and Cohen, 1973). In contrast, salsolinol was observed to produce a dose-dependent biphasic hypothermic-hyperthermic response upon intraventricular injection (Hamilton

and Hirst, 1974). In a recent test salsolinol reduced the number of responses of rats in a food-reinforcement task (Hymowitz and Brezenoff, 1978). Some isoquinolines substituted at positions 6 and 7, particularly the methoxyl compounds, decreased motor activity in mice and generated tremors and convulsions (Hjort et al., 1942; Teitel et al., 1974). Other behavioural responses have been observed for TIQs as they relate to alcohol or opiate actions, but these findings will be elaborated in a following section. Beta-carbolines were shown to produce in rats a conditioned behavioural reflex (Taborsky and McIsaac, 1964). A discussion on the prominent effects of tetrahydro-beta-carbolines in alcohol consumption will be presented shortly.

8. Isoquinolines as False Transmitters

The concept that a substance other than the natural transmitter may be released from a nerve and function in the manner of the normal neuronal agent has been reviewed by Kopin (1968). These "false transmitters" must have the same physiological properties as the natural transmitter, yet the potency may differ greatly. The mechanisms of uptake, storage and release of an imitator must coincide with the processes operating for the native substance. Based upon many findings in his laboratory, Cohen has envisioned a role for TIQ alkaloids as false transmitters in adrenergic neurons (Cohen, 1973). Mytilineou et al. (1974) demonstrated that norsalsolinol was taken up and released by sympathetic nerves leading to the iris, with typical sympathetically-mediated iris contraction and eyeball protrusion being effected. The way

in which norsalsolinol and the adrenaline-acetaldehyde adduct mimicked noradrenaline activity in sympathetic systems was suggestive of a false transmitter role (Osswald *et al.*, 1975; Simpson, 1975). It has also been hypothesized that THP or some other dopamine-related TIQ may be operating in such a manner to produce some of the effects observed in Parkinsonism (Sourkes, 1971; Sandler, 1973).

9. The Role of Isoquinolines in Parkinsonism

One neurological phenomenon accompanied by striatal dopamine deficiency is that of Parkinson's disease (Hornykiewicz, 1966). The therapeutic approach to the problem has been the administration of several grams of L-DOPA (Birkmayer and Hornykiewicz, 1961), the dopamine precursor which is able to cross the blood-brain barrier (De la Torre, 1973), or L-DOPA in combination with a peripheral decarboxylase inhibitor (Bianchine, 1976). Recognizing that a high level of L-DOPA is present centrally upon such administration and that L-DOPA and its catecholamine metabolites can condense with aldehydes to form TIQs, Sourkes (1971) postulated a role in Parkinsonism for an L-DOPA-based TIQ, THP in particular. Dopamine and the deaminated aldehyde produced by the action of MAO possibly combine in the striatum or some associated area to form THP. Sandler *et al.* (1973) suggested that THP, which they detected in the urine of Parkinson patients who had ingested L-DOPA and ethanol, perhaps was responsible for some of the central or peripheral effects observed in patients undergoing L-DOPA treatment. Salsolinol has also been detected in the urine of patients on L-DOPA

(Sandler *et al.*, 1973; Nijm *et al.*, 1977), and it, too, may have fulfilled a role in the L-DOPA therapy. Even in the absence of ethanol, THP formation was detected following L-DOPA administration to rats (Turner *et al.*, 1974). When tested clinically, THP was observed to exacerbate the tremors attendant upon Parkinsonism (Dordain *et al.*, 1974). The observations noted upon salsolinol or THP administration to a preparation of dopamine receptors suggested to the authors (Dougan *et al.*, 1975) that the isoquinolines may function in the akinesia paradoxica, or "on-off" response, occurring during L-DOPA therapy in Parkinsonism (Sweet *et al.*, 1975; Bianchine, 1976).

10. Alteration of Divalent Calcium Levels

It was reported a few years ago that salsolinol depleted calcium from discrete regions of rat brain (Ross, Medina and Cardenas, 1974). Naloxone prevented the isoquinoline-induced depletion. In later work the authors reported dose-dependent calcium-binding inhibition by salsolinol at high affinity sites on synaptic membranes (Ross, 1978). Again, naloxone protected against the action demonstrated by salsolinol. These findings were important in terms of defining basic features of alcohol and opiate addiction, as will be discussed in a subsequent section.

11. Inhibition of Neurotransmitter Regulatory Enzymes

Neurotransmitter formation and degradation rely upon the actions of particular enzymes. In the metabolism of catecholamines and serotonin the enzymes monoamine oxidase (MAO) and catechol-O-methyl

transferase (COMT) appear prominently. Within the past decade isoquinolines and beta-carbolines were shown to have the ability to inhibit MAO and COMT, as revealed in studies performed _in vitro_ and _in vivo_.

Yamanaka (1971) demonstrated that salsolinol could inhibit rat brain and liver MAO competitively in homogenates of the tissues. Later it was shown that both THP and salsolinol were inhibitors of MAO (Collins _et al_., 1973). Evidence was presented for enzyme inhibition _in vivo_ in tests with norsalsolinol. The quantity of deaminated products of noradrenaline in murine heart sympathetic nerves was significantly increased after treatment with norsalsolinol (Cohen and Katz, 1975). More recently, these authors noted that in the isolated heart preparation norsalsolinol and salsolinol were more potent than THP as inhibitors of MAO action or noradrenaline (Katz and Cohen, 1976). Beta-carbolines also displayed activity as competitive inhibitors of MAO _in vitro_ (Ho _et al_., 1968; Dajani and Saheb, 1973; Buckholtz and Boggan, 1976).

The separation of the serotonin- and noradrenaline-preferring form (type A) of MAO from the less specific phenethylamine-preferring form (type B) has been achieved (Yang and Neff, 1973). Salsolinol and a tetrahydroxyberbine were found to be competitive inhibitors of type A MAO, whereas THP was a competitive, though nonspecific, inhibitor of rat brain MAO (Meyerson, McMurtrey and Davis, 1976). In another study on rat brain MAO unsubstituted tetrahydro-beta-carboline and a 6-methoxyl derivative were potent competitive

inhibitors of type A but showed little effect on type B (Meller *et al.*, 1977).

The activity of COMT was altered in rat liver and brain tissues by several isoquinolines. Collins *et al.* (1973) demonstrated that salsolinol and THP were competitive inhibitors of dopamine-O-methylation in rat liver homogenates. Following acute treatment with salsolinol or THP, rat brain COMT function was significantly depressed 2 hr. after injection (Giovine, Renis and Bertolino, 1977). By the 20th day after treatment the COMT activity in THP-injected animals was significantly elevated above control values; in salsolinol-injected animals, significantly lowered. Incubation of rat brain enzyme preparations of MAO and COMT with salsolinol and THP had previously shown that the former isoquinoline was the more effective inhibitor of the metabolism of dopamine and serotonin (Giovine, Renis and Bertolino, 1976). In another report Smissman *et al.* (1976) demonstrated that 2- and 4-substituted derivatives of norsalsolinol were effective inhibitors of COMT in mouse hearts *in vivo*.

Tyrosine hydroxylase, the enzyme involved in the rate-controlling step of catecholamine synthesis, has been investigated for possible isoquinoline-induced changes. Collins (1977) reported that salsolinol and other catechol TIQs, excluding THP, inhibited tyrosine hydroxylase *in vitro*.

C. Metabolism of Tetrahydroisoquinolines

Based upon recent findings, it appears that TIQs are capable of undergoing O-methylation. Other possible metabolic processes known to operate for catecholamines have not been extensively examined. Furthermore, it is unknown whether the TIQs excreted into urine (Nijm et al., 1977; Sandler et al., 1973) were the products formed initially or whether they resulted from sets of enzymatic or nonenzymatic reactions.

Collins et al. (1973) demonstrated that salsolinol was O-methylated by a rat liver COMT preparation. The maximum velocity of the reaction approximately tripled that of dopamine O-methylation and doubled that of noradrenaline O-methylation. In the presence of a COMT preparation derived from rat brain salsolinol appeared to be a slightly better substrate for O-methylation than dopamine, judging by the Km and velocity values calculated (Giovine et al., 1976). Hamilton et al. (1978) have detected an O-methylated congener of salsolinol in the striata of mice exposed to ethanol vapour. However, the sequence of reactions leading to the formation of the O-methylsalsolinol is not known. The action of COMT on salsolinol, the conjugation of acetaldehyde with O-methylated dopamine or some other series of events may have been involved. Recently, it was reported that administration of a COMT inhibitor prolonged the half-lives of THP and salsolinol in rats (Melchior, Mueller and Deitrich, 1978).

Two laboratories reported on the ability of norsalsolinol and some of its methylated congeners to act as substrates for COMT *in vitro* (Creveling *et al*., 1972; Smissman *et al*., 1976). For norsalsolinol, the 7-hydroxyl group appeared to be preferentially methylated by COMT, rather than the 6-hydroxyl group (Creveling *et al*., 1972). The ionic environment as well as the pH were important in determining the 6:7 O-methylation ratio.

A variety of metabolic pathways seems to be available to THP. In comparison with salsolinol, dopamine and noradrenaline, THP displayed the best kinetic profile in O-methylation by COMT homogenates from rat liver (Collins *et al*., 1973) and rat brain (Giovine *et al*., 1976). It has also been shown that THP can be metabolized to tetrahydroprotoberberine alkaloids *in vivo* in rats or *in vitro* in the presence of rat brain or liver homogenates (Cashaw *et al*., 1974). The authors showed that these same berberine products were formed in humans on L-DOPA therapy. The insertion of a "berberine bridge" onto THP is known to be a step in the formation of more complex alkaloids in opium plants (Kirby, 1967).

The administration to Parkinsonian patients of L-DOPA with or without the peripheral decarboxylase inhibitor carbidopa has yielded one further class of THP compounds. A carboxylic acid derivative and an O-methylated carboxylic acid derivative of THP were found in Parkinsonian patients and in rats both given L-DOPA (Coscia *et al*., 1977). The authors presented evidence in support of the possibility that methylation by COMT preceded any condensation involved.

Rubenstein and Collins (1973) illustrated that the TIQs formed from noradrenaline condensation with acetaldehyde or formaldehyde were better substrates for COMT than noradrenaline itself. In rat brain and liver homogenate studies, use of the COMT inhibitor pyrogallol resulted in significant attenuation of the isoquinoline O-methylation. Also, more complex aldehyde-based conjugates of dopamine and noradrenaline were again better substrates for COMT *in vitro* than the parent amines (Ho *et al*., 1974).

Dajani and Saheb (1973) presented a chart of proposed and defined pathways of beta-carboline metabolism. It appears that O-methylation is the most important step taken initially.

D. Pharmacology of Ethanol

1. Biosynthesis of Ethanol

The ability of microorganisms to synthesize ethanol has been exploited from the time of early civilization until the present era. Through technological advances man continues to elevate zymurgy to sophisticated levels (Stanier, Doudoroff and Adelberg, 1970).

In the livers and other tissues of rats, rabbits and humans, ethanol has been found in micromolar quantities after the ingestion of an ethanol-free diet (McManus, Contag and Olson, 1960). Following the suppression of intestinal bacteria the authors observed no significant change in the amounts of ethanol in each 100 g of a given tissue. According to the researchers, it appeared that at least some of the ethanol arose upon hepatic oxidation of pyruvate.

Tests performed on fasting and nonfasting individuals indicated that 1 g or more of ethanol was formed daily in patients displaying normal gastrointestinal function (Blomstrand, 1971).

2. Metabolism of Ethanol

Subsequent to rapid absorption and uniform distribution into all fluids and tissues of the body, ethanol undergoes oxidative metabolism. As the major features of ethanol oxidation have been extensively reviewed elsewhere (Jacobsen, 1952; Hawkins and Kalant, 1972), only those features pertinent to the present debate are outlined in the following discourse.

A limited number of pathways are followed in ethanol metabolism. No matter which route is taken, the initial step involves oxidation to acetaldehyde. The aldehyde is rapidly oxidized to acetic acid. Conversion to acetyl coenzyme A permits entry of the carbon atoms into the citric acid cycle or synthesis of tissue components.

Of greatest importance to ethanol metabolism is the activity of alcohol dehydrogenase (ADH), which is located mainly in the soluble region of hepatocytes. Raskin and Sokoloff (1972) examined a variety of rat tissues for ADH activity and discovered that the brain was one of the organs capable of oxidizing ethanol by means of ADH. Subsequent tests revealed that the time course of the development of tolerance to ethanol paralleled increases in brain ADH activity (Raskin, 1973). However, the search for indications of ADH activity in isolated perfused rat brains has

proved futile (Mukherji et al., 1975), necessitating further work into neuronal ADH and its status as a metabolizer of ethanol. Both zinc and nicotinamide adenine dinucleotide (NAD) are necessary cofactors for effective oxidation by ADH. How NAD, ADH and alcohols interact in the form of binary and ternary complexes to generate the corresponding aldehydes was originally outlined by Theorell and Chance (1951). The rate-limiting reaction in the metabolism of ethanol is the regeneration of NAD from its reduced form (NADH) produced during ethanol oxidation. Maximal activity of ADH is apparent at low blood ethanol concentrations (Von Wartburg, 1971) and in some cases solely determines the rate of ethanol elimination (Crow, Cornell and Veech, 1977).

Attempts have been made to assign a physiological role to ADH. It has been suggested that the enzyme serves to metabolize the ethanol present in the gastrointestinal tract (Krebs and Perkins, 1970). Other work has focussed on retinol, the alcoholic form of vitamin A, as a substrate candidate for ADH (Raskin, Sligar and Steinberg, 1976). Because ADH lacks specificity, several endogenous compounds or classes of compounds may eventually be found to be the true substrates.

Inhibitors of ADH have been used in many experiments designed to clarify the mechanism of action of ethanol. In this regard, a potent class of compounds is the pyrazoles, which appear to complex with ADH and the cofactors (Orth, 1968). Using mouse liver ADH Goldstein and Pal (1971) demonstrated that pyrazole was a competitive

inhibitor of the enzyme. The authors also showed that pyrazole had a half-life of 10 hr. in mice. In rats it was observed that pyrazole slowed the elimination of ethanol from blood and the expiration of radiolabelled carbon dioxide following the administration of radiolabelled ethanol (Goldberg and Rydberg, 1969). Again, a competitive type of inhibition was apparent.

Behavioural ramifications of pyrazole administration have been explored. Pyrazole and 4-methylpyrazole significantly impaired coordination in rats in the presence and absence of ethanol (Goldberg et al., 1972; Rydberg and Neri, 1972) and produced a catatonic state (Macdonald, Marselos and Nousiainen, 1975). In mice, pyrazole increased the amount and duration of locomotor activity displayed upon withdrawal from ethanol, and produced hypothermia and weight loss whether given alone or prior to ethanol (Littleton, Griffiths and Ortiz, 1974). Disturbance of catecholamine concentrations was considered as a possible mechanism in the behavioural responses to pyrazole. However, pyrazole (68 mg/Kg) did not significantly alter catecholamine levels in either central (Littleton et al., 1974) or peripheral sites (MacDonald et al., 1975).

Apparently, pyrazole has the ability to affect not only ADH but also other enzymes. For instance, Powis and Grant (1976) showed that in rats hepatic microsomal hydroxylation of aniline and demethylation of aminopyrine were increased by pyrazole administration.

Oxidative enzymes other than ADH have been invoked in the metabolism of ethanol. Currently, active debates abound as to the

relevance of the various enzymes in ethanol oxidation. The ubiquitous catalase has recently been promoted as an enzyme responsible in part for ethanol catabolism (Thurman and Brentzel, 1977). But, most of the fury has centred around the possible involvement of a liver microsomal ethanol-oxidizing system (MEOS). This subcellular fraction has been hypothesized to be responsible for the enhanced rate of ethanol metabolism recorded after chronic ethanol intake (Teschke et al., 1977). On the other hand, MEOS activity was not detected in ethanol-tolerant rats at the time of maximum ADH inhibition by pyrazole (Kalant, Khanna and Endrenyi, 1975).

Aldehyde dehydrogenase (AldDH) is the enzyme class responsible for conversion of acetaldehyde to acetic acid. Although other enzymes have the ability to use acetaldehyde as a substrate AldDH is the most important enzyme metabolizing the acetaldehyde generated during ethanol metabolism (Westerfeld, 1955). By far the most active tissue source of AldDH is the mitochondrial matrix of liver (Raskin and Sokoloff, 1972), but significant activity has also been found in the brain (Mukherji et al., 1975). Its low level in human placenta may impart toxicological significance to maternal ethanol ingestion (Kouri, Koivula and Koivusalo, 1977). The requisite cofactor for AldDH activity is NAD (Erwin and Deitrich, 1966), the regeneration of which appears to be linked with pyruvate reduction (Ridge, 1963).

The enzymatic activity of AldDH has been altered in the presence of various drugs. Short-term phenobarbital administration noticeably increased liver AldDH activity in mice, a response of possible significance in the development of tolerance to ethanol in the presence of barbiturates (Redmond and Cohen, 1971). Inhibition of AldDH in rats has been observed after the administration of a variety of compounds, including the COMT inhibitor pyrogallol (Rubenstein, Collins and Tabakoff, 1975) and the decarboxylase inhibitor benserazide given in the presence or absence of L-DOPA (Messiha, 1977). Of great clinical interest is the inhibition of AldDH by disulfiram treatment. For many years the enzymatic processes and behavioural sequelae apparent after the administration of disulfiram has been the focus of much attention (Hald, Jacobsen and Larsen, 1948; Deitrich and Erwin, 1971; Saint-Blanquat and Derache, 1976; Kitson, 1977). The reports suggest that disulfiram is converted _in vivo_ to diethyldithiocarbamate which then proceeds to inhibit AldDH irreversibly. Judging by the blockade of the enzymatic activity after cycloheximide treatment, protein renewal is necessary for reactivation of AldDH (Deitrich and Erwin, 1971).

The administration of disulfiram either alone or given prior to ethanol has generated various behavioural responses. In an ethanol-preferring strain of mice the blood acetaldehyde increase created by disulfiram-ethanol treatment was accompanied by a significant decrease in voluntary alcohol consumption (Schlesinger, Kakihana and Bennett, 1966). Spontaneous locomotor activity underwent a dose-dependent attenuation in the presence of disulfiram (Moore,

1969). In human subjects the administration of disulfiram has been widely used as a deterrent to drinking in alcoholic individuals, but the therapy has not been free of unpleasant or injurious effects (Fried, 1977; Prigatano, 1977; Ritchie, 1975). Furthermore, successful deterrence from drinking appears to rely heavily on psychological factors (Kitson, 1977). The pharmacological agent implicated in the production of the effects observed after the intake of alcohol and disulfiram is acetaldehyde, which will be discussed in greater detail in a following section.

Behavioural depression produced by disulfiram in rats and mice was accompanied by lowered noradrenaline levels in the brain (Goldstein and Nakajima, 1967; Moore, 1969). Dopamine-beta-hydroxylase (DBH) is the enzyme responsible for converting dopamine to noradrenaline, and it appears that this enzyme can be inhibited by disulfiram (Goldstein et al., 1964). Copper, a necessary cofactor in the functioning of DBH, was chelated by disulfiram, thereby inactivating the enzyme (Deitrich and Erwin, 1971; Hald et al., 1948).

3. Biochemical and Behavioural Correlates of Altered Ethanol Metabolism

Many different chemicals have been found to change the rate of ethanol metabolism. Little is known of the mechanisms responsible for the alterations. Several of the compounds have been evaluated for possible clinical benefit.

The administration of D-penicillamine, a sulfhydryl amino acid, diverted the acetaldehyde generated during ethanol metabolism

into the formation of a thiazolidine carboxylic acid (Nagasawa et al., 1975). Other sulfhydryl-containing amino acids neither promoted the carboxylic acid formation nor altered the blood acetaldehyde levels attained after treatment with disulfiram and ethanol (Nagasawa et al., 1977). The authors reported no significant change in blood ethanol levels following the administration of D-penicillamine.

It was previously mentioned that pyrazoles inhibit ADH. In this way ethanol metabolism is decreased. Therefore, the implication was made that pyrazoles might be used in human patients to generate sobriety gradually (Lester, Keokosky and Felzenberg, 1968). In contrast with this tactic has been the attempt to hasten ethanol metabolism through the use of fructose or a related sugar (Rawat, 1977). By encouraging the regeneration of NAD from NADH, the reduction of fructose to glycerol has been advocated as a means of lowering blood ethanol levels. This hypothesis has been detailed elsewhere (Deitrich, 1976). It was noted that although the administration of fructose led to a marked increase in blood ethanol clearance, no significant change occurred in blood acetaldehyde levels (Rawat, 1977). It was concluded that such a finding was indicative of the relative insensitivity of AldDH to changes in the redox state of NAD.

Certain inhibitors of MAO have been found to alter the blood acetaldehyde levels attained following ethanol intake. Pargyline increased the blood concentration of acetaldehyde in mice and in rats (Cohen, MacNamee and Dembiec, 1975). The mechanism responsible

for the change was not demonstrated. However, it has more recently been revealed that pargyline can inhibit a low-Km AldDH (Lebsack, 1977). Pargyline was observed to increase acetaldehyde levels in mice to a greater degree than did another MAO inhibitor, nialamide (Sanders et al., 1977). A more pronounced decrease in ethanol preference was achieved with pargyline as compared with nialamide, but an association between this response and the acetaldehyde level changes was not explored further. Although the extent of MAO inhibition observed with nialamide was significantly greater than with pargyline (Sanders et al., 1977), the changes in the blood level of acetaldehyde appear to have little connection with MAO inhibition (Cohen et al., 1975). Other studies conducted on pargyline-ethanol interactions suggested that the behavioural depression observed in mice after the combined treatment resulted from a pargyline-induced slowing of ethanol metabolism as well as some pargyline-induced change in neuronal sensitivity to ethanol (Collins, Lebsack and Yeager, 1976).

Chronic ethanol administration has been well documented as a mode of increasing ethanol catabolism. In mice, chronic ethanol consumption early in life produced an increase in hepatic ADH activity as compared with water-imbiding subjects (Dippel and Ferguson, 1977). In rats it appears that the rate of ethanol metabolism is dependent on the strain of rat used in a given test, the duration of imbibition and the concentration of ethanol. It was reported that the activity of ADH did not vary among strains, yet the ability of the liver to metabolize ethanol did differ

significantly (Videla and Israel, 1970; Koivula and Lindros, 1975). Lower activities of mitochondrial AldDH were accompanied by higher blood acetaldehyde levels (Koivula and Lindros, 1975). Increases in hepatic uptake of oxygen have been observed after chronic ethanol consumption (Thurman and Scholz, 1976). According to the authors, an increased oxygen uptake may perhaps be translated simply into increased ethanol oxidation. Surely, many factors operate to enhance the metabolism of alcohol after chronic intake. Components of diet, age and stress factors and the availability of ADH-independent pathways of ethanol catabolism have been implicated in the enhancement (Cederbaum _et al_., 1977; Israel _et al_., 1977; Thurman and Brentzel, 1977). Once the fatty content of the liver has reached a particular level after chronic ethanol ingestion, hepatic enzymes, including ADH, probably begin to be adversely affected (Videla and Israel, 1970; Dippel and Ferguson, 1977).

In human subjects the chronic ingestion of alcohol apparently leads to more rapid metabolism of ethanol, an interpretation based upon the observed increases in blood acetaldehyde concentrations (Truitt, 1971; Korsten _et al_., 1975). At a particular blood alcohol level (24 mM) the concentration of acetaldehyde began to decline, suggesting desaturation of an enzyme oxidizing ethanol or damage to mitochondrial AldDH (Korsten _et al_., 1975). Because the zero-order kinetics and low K_m of liver ADH indicate saturation of the enzyme at ethanol concentrations in tissues of 2 to 3 mM (Raskin, 1975), the invoked "enzyme desaturation" hypothesis implies the involvement of some enzyme other than ADH.

The influence of genetic factors on ethanol metabolism and on the associated behavioural changes has been under investigation in many laboratories. It was demonstrated that differences in sex and strain were implicated in the differences observed in ethanol elimination and duration of the loss of righting reflex in mice (Collins et al., 1976). The authors reported that variation in ADH activity was not detected between strains or between sexes. Similarly, in an examination of other strains of mice differing in the duration of ethanol-induced narcosis, no statistical difference in ADH or AldDH was apparent (Heston et al., 1974). However, in some strains of mice variation in ADH and AldDH activity, enough to produce variation in blood alcohol clearance, was instrumental in creating differences in the duration of ethanol narcosis (Sheppard, Albersheim and McClearn, 1968, 1970; Damjanovich and MacInnes, 1972; Berger and Weiner, 1977). In turn, greater preference for ethanol was associated with the development of greater tolerance to some of the centrally depressant effects of ethanol (Schneider et al., 1973). Moreover, the strain-associated variability in sensitivity to the central effects of ethanol was not translatable to the narcosis induced by pentobarbital, even though the probable critical site of action, the reticular activating system, was implicated in both cases (Randall and Lester, 1974; Siemens and Chan, 1974).

Genetic factors in human populations have been examined in studies on ethanol metabolism and related behavioural changes. As human alcohol consumption is the result of many interacting physical and social factors, it is not surprising that apparently conflicting

evidence has appeared for the involvement of heredity. Although the rate of ethanol metabolism has been found to differ among certain Caucasian, Chinese and Canadian native populations, the variability plays only a small part in the differences in susceptibility to the effects of alcohol (Reed *et al.*, 1976). Although the blood ethanol and acetaldehyde levels differed among the groups, ADH and AldDH activities were not examined by the authors for their role in producing the variations. However, it is known that in the Japanese population the presence of an "atypical" liver ADH is responsible for marked differences in vascular responses to ethanol consumption (Deitrich, 1976). The need for further research into racial factors as they relate to alcoholism was explored in a recent seminar (Seixas, 1978).

Ethanol intake serves to affect hepatic structure and function adversely. Because the metabolic processes in operation in the liver are so crucial to biochemical reactions in all other organs, the detrimental effects of alcohol on liver function have far-reaching implications in the body.

As a consequence of lowered NAD/NADH ratios arising from ethanol oxidation, all reactions dependent on the normal redox state of the liver are significantly affected (Forsander, 1970; Hawkins and Kalant, 1972). Fatty acid oxidation is one of the processes decreased. Hepatotoxicity becomes apparent once the free fatty acid concentration is elevated as a result of both the attenuated oxidation and the alcohol-induced mobilization of the free fatty

acids from other tissues. Chronic intake of alcohol produces a gradually increasing infiltration of fat into the liver, a condition which usually proceeds to hepatitis and then to cirrhosis and extensive heptatic malfunction. How ethanol produces the various degrees of liver pathology and how dietary factors may be implicated in the problem have been questioned and explored in great detail by Lieber (1975).

Ethanol-induced redox changes in the metabolism of certain biogenic amines have been widely reported and well reviewed (Davis and Walsh, 1971; Hawkins and Kalant, 1972). Acute administration of ethanol led to increased excretion of the reduced form of the metabolite of noradrenaline and decreased excretion of the oxidized metabolites of dopamine and noradrenaline in rats and humans (Davis _et al._, 1967b; Bertani _et al._, 1969). Chronic ethanol administration widened the disparity in the amounts of reduced and oxidized adrenergic metabolites excreted (Ogata _et al._, 1971). Output of the metabolites of dopamine displayed varied patterns of excretion in the chronic testing. In rat liver preparations ethanol elevated the concentration of the reduced form of dopamine (Tank, Weiner and Thurman, 1976). In man elevation of the urinary content of the reduced metabolite of serotonin occurred at the expense of the oxidized form after acute ethanol intake (Davis _et al._, 1967a; Feldstein _et al._, 1967). Apparently, the change in the metabolism of serotonin may have occurred mainly in the liver rather than in the brain, judging by the responses observed in studies performed with tissue slices (Eccleston, Reading and Ritchie, 1969). It has been proposed that

the shift in the NAD/NADH relation arising from ethanol intake is the sole factor responsible for the shift in the metabolism of serotonin (Feldstein et al., 1967), but it has been argued that other parameters must be considered as well (Davis and Walsh, 1971). The presence of a beta-hydroxyl group on the parent amine (Turner et al., 1974) and the inhibition by ethanol of the active removal of the oxidized product from cerebrospinal fluid (Tabakoff, Bulat and Anderson, 1975) are factors of possible influence in the alteration of the metabolic patterns observed for catecholamines and serotonin, respectively. Furthermore, the competitive inhibition of AldDH by the product of ethanol metabolism, namely, acetaldehyde, may prove to be the mediator responsible for the altered amine metabolism (Walsh, Truitt and Davis, 1970).

Acute administration of ethanol has been found to attenuate the activity of hepatic microsomal metabolism of drugs (Rubin and Lieber, 1971; Khanna et al., 1978). On the other hand, chronic consumption of ethanol enhanced the activity of the microsomal system (Lieber, Rubin and DeCarli, 1971; Kalant et al., 1976). These tests performed with pharmacologic agents, such as, meprobamate and pentobarbital, bring to mind the problems of dosage regulation associated with the combination of drug therapy and alcohol intake and the creation of adverse effects.

5. Neural Effects of Ethanol

a. Introduction

By altering biochemical features of the central nervous system, ethanol generates changes in behaviour (Deitrich, 1976; Myers, 1978). Both of these levels of neuronal perturbation have been subjected to intensive investigation in the past and continue to be enthusiastically examined. However, as quickly as the mysteries of alcohol activity unfold, more and more clues are presented for analysis. The neuronal characteristics currently being scrutinized are considered in the discourse to follow. Only through integrated study of these various expressions of alcohol-induced neuronal change will valid conclusions arise (Kalant, 1975).

b. Effects on Membranes

The anaesthetic actions of ethanol, a compound simple in structure, have been considered to arise from neuronal membrane disruption in a manner reminiscent of the actions of other known simple anaesthetics. Membrane fluidity changes rather than alterations at specific sites on the membrane have been implicated in the central depressant effects of the anaesthetics. Recently, it was demonstrated that ethanol increased the fluidity of murine synaptosomal and mitochondrial membranes, but not that of myelin membranes, in a dose-dependent fashion (Chin and Goldstein, 1977). Also, the fluidization of presynaptic membranes in a cholinergic preparation was suggested to be operating in the development of tolerance to the effect of ethanol on post-tetanic potentiation (Traynor et al., 1977).

Using ethanol-tolerant rats, changes in the membrane fluidity of cholinergic nerve terminals were hypothesized in an indirect study of the possibility. The authors reported that phrenic nerve terminals obtained from ethanol-tolerant rats demonstrated a lower relative miniature end-plate potential frequency upon exposure to ethanol than did terminals obtained from sucrose-fed rats (Curran and Seeman, 1977).

In the fluidization theory presented in the preceding paragraph changes in the hydrophobicity of the membranes have been suggested. Other work has shown that perhaps also protein changes occur in an ethanol-tolerant state. Membrane protein sites of calcium binding were exposed on synaptic plasma membranes removed from rats chronically fed low doses of ethanol (Ross, Kibler and Cardenas, 1977). This finding concurs with the demonstration that in mice low doses of ethanol increased the ability of the synaptosomal membrane components to transport calcium into the nerve terminal (Sun, 1976). Higher doses of ethanol progressively hampered calcium transport.

c. Electrophysiological Responses

In simple models of neuronal and ganglial activity ethanol produced various degrees of enhancement or depression of post-synaptic spike generation (Faber and Klee, 1977). Tests conducted in ole animals were somewhat more informative. Iontophoretic application of ethanol into discrete brain regions of rats demonstrated differences in sensitivity to the effects of the drug. The discharge frequency of neurons located in the lateral hypothalamus or thalamus was

increased significantly, whereas cells of the cerebral cortex were less vulnerable to the activity of ethanol (Wayner, 1973; Wayner, Ono and Nolley, 1975). It was suggested that the interneurons of the lateral hypothalamus were particularly susceptible to ethanol-induced increases in discharge frequency. Studies conducted on cats demonstrated that ethanol produced dose-dependent changes in cerebral cortical electrical activity which were paralleled by dose-dependent changes in the depth of narcosis (Horsey and Akert, 1953). Thalamic and caudate nuclear electrical activities were less affected. Subsequently, Himwich _et al._ (1966) showed in an investigation of reflex activity that in the cat neurons of the midbrain reticular formation were more sensitive to the depressant effects of ethanol than were neurons of the primary somatosensory cortex. In mice, only indirect evidence for altered electrical activity of the brain is available. It was observed that ethanol displayed anti-convulsant activity at high doses given acutely and lowered the seizure threshold upon sudden termination of chronic administration (McQuarrie and Fingle, 1958).

Disruptions of normal electrical activity of human brain have been witnessed after alcohol consumption. In alcoholics the total sleep time was reduced during intoxication, and the proportion of time spent in various stages of sleep was significantly altered in the intoxicated state and in withdrawal (Gross _et al._, 1973). In nonalcoholic individuals sleep-pattern disruption was apparent following intake of alcohol at bedtime (Johnson, Burdick and Smith, 1970). The abnormalities in sleep were attributed at least in part

to malfunctioning of neuroamines, serotonin in particular, which operate to establish sleep patterns (Jouvet, 1969).

d. Effects on Cyclic Nucleotides

The ethanol-induced alteration in redox potential has been implicated as a factor in changing the nucleotide metabolism and activity in various tissues (Rawat, Kuriyama and Mose, 1973). In the livers of rats exposed chronically to ethanol intake the amount of ATP was reduced (Gordon, 1977), as it was in the brains of mice given similar treatment (Rawat et al., 1973). Also, chronic ethanol administration significantly enhanced the activities of adenylate cyclase in murine brain and liver and cyclic AMP-dependent protein kinase in murine brain synaptosomal fractions (Kuriyama, 1977). But, the author observed no change in activity for either cerebral or hepatic phosphodiesterase. In cerebrospinal fluid taken from alcoholics after ethanol consumption the concentration of cyclic AMP was lower than that prior to ingestion (Orenberg et al., 1976b). Acute ethanol tests revealed diverse changes in adenylate cyclase activity in discrete brain areas in mice (Orenberg, Renson and Barchas, 1976a) and in rats (Volicer et al., 1977).

The activity of the enzyme $(Na^{+}+K^{+})$-ATPase, which is responsible for the active transport of the two cations across cell membranes, was altered in tests performed in vitro and in vivo. Ethanol demonstrated competitive inhibition of potassium ion transport and noncompetitive inhibition of sodium ion transport in homogenates of murine cerebral cortex (Lin, 1976). Synaptosomal plasma membranes

prepared from squirrel monkey cortex were another site of ethanol-induced inhibition of the enzyme (Sun, 1976). Also, cation transport inhibition was demonstrated in rat brain slices after chronic ethanol intake (Israel, 1970). However, enhanced enzyme activity was observed for the $(Na^+ + K^+)$-ATPase located in rat liver (Gordon, 1977).

e. Effects on Cerebral Synthesis of Proteins

Briefly, the chronic ingestion of ethanol by rats and mice resulted in attenuation of protein synthesis in brain tissue (Noble and Tewari, 1975; Tewari, Golstein and Noble, 1977). The events mediated by the ribosomal nucleic acids and the enzymes associated with their actions were inhibited in the rodents during chronic intoxication and to an even greater degree during withdrawal. Accordingly, the development of structural brain damage in alcoholism may be of considerable importance.

f. Effects on Levels of Putative Neurotransmitters

Experimentation into the effects of ethanol on the central levels of neuroamines and other transmitters burgeoned rapidly in recent years. In fact, the expansion occurred so hastily that the resulting plethora of findings is fraught with contradictions. Reviewers of the subject have attested to the congestion of information (Hawkins and Kalant, 1972; Deitrich, 1976; Tabakoff, 1977; Myers, 1978). Among the studies so many different methodologies were employed that few definitive results have surfaced.

Researchers do appear to concur that noradrenaline turnover was decreased by acute ethanol treatment and was increased either during chronic exposure or upon withdrawal (Hunt and Majchrowicz, 1974; Pohorecky, 1974; Thadani and Truitt, 1977). Absolute levels of noradrenaline were increased during withdrawal but not while ethanol was being administered (Pohorecky et al., 1978). Acute oral consumption (Carlsson et al., 1973) or inhalation (Ortix, Griffiths and Littleton, 1974) of ethanol lowered the amount of dopamine present in murine brain. Following chronic administration of ethanol to mice a decrease in the turnover of dopamine was observed; following withdrawal, an increase (Hunt and Majchrowicz, 1974). Although an ethanol-induced enhancement of noradrenaline and dopamine excretion had been reported earlier, whether the source of the amines was central or else peripheral was not established (Anton, 1965).

Research on the changes in the state of serotonin in brain has presented the most diagreement. Kuriyama, Rauscher and Sze (1971) and Frankel et al. (1974) observed no alteration of serotonin turnover upon acute administration of ethanol. However, in chronic experiments the reported increase in turnover of serotonin (Kuriyama et al., 1971) did not receive support from Frankel et al. (1974).

Acetylcholine located in the brain was affected by ethanol in a variety of tests. Although the acetylcholine release from rat cerebral cortical slices was significantly attenuated, the total content of acetylcholine in the slices was not altered (Kalant, Israel and Mahon, 1967). Neither was any change in the activity of

choline acetyltransferase reported. Chronic ethanol administration served to increase acetylcholine uptake into murine cortical slices, while acute treatment inhibited acetylcholine release (Carson, Jenden and Noble, 1975). Fasting prior to ethanol consumption led to a greater increase in acetylcholine content in homogenates of whole brain of rats (Brown, Post and Mallov, 1977).

Reports on change in the level of GABA by ethanol are contradictory. Chronic administration of ethanol produced a decrease, an increase or no change in GABA levels, depending upon the experiment conducted (Patel and Lal, 1973; Rawat, 1974). Acute ethanol administration to mice elevated the GABA level in brain (Rawat, 1974).

g. Behavioural Expression of Neural Effects

Psychological testing conducted on human alcoholics and naive subjects has revealed that ethanol consumption is associated with marked impairment of mental function (Barry, 1974; Parsons, 1977). Sensory and motor tasks are poorly performed. Learned behaviour is especially impaired (Walker and Freund, 1973). In the advanced stage of alcoholism neurologic damage appears in the form of psychiatric syndromes. The visual impairment and memory loss expressed in Korsakoff's disease and the confused state and central histopathology of Wernicke's encephalopathy are considered to be sequelae of not only chronic abuse but also of the nutritional deficits so prevalent in alcoholism (Dreyfus, 1973; Butters et al., 1977; Vogel, 1977).

In animals there is evidence that neuroamines participate in the behavioural effects observed after acute or chronic alcohol consumption. Studies performed on ethanol preference, its central depressant effects and the phenomena of dependence and tolerance present valuable information on neuroamine involvement.

Whether catecholamines act to determine the extent of alcohol consumption has been investigated in several laboratories (Myers, 1978). A reduction in cerebral catecholamine levels in rats after treatment with alpha-methyl-p-tyrosine or 6-hydroxydopamine led to a reduction in alcohol imbibition (Myers and Veale, 1968; Myers and Melchior, 1975). Confining noradrenaline depletion to the forebrain resulted in increased ethanol intake (Kiianmaa, 1975), suggesting a possible malfunction in the reward mechanism. In other tests the inhibition of the enzyme dopamine-beta-hydroxylase (DBH) in rats resulted in a marked decline in alcohol consumption (Amit, Levitan and Lindros, 1976). Humans who display relatively high DBH activity in their blood are capable of attaining higher blood ethanol levels without suffering as much drunkeness (Ewing, Rouse and Mills, 1975). However, it must be kept in mind that the relative roles of dopamine and noradrenaline in the effects observed in the DBH studies have not been established.

Myers and Melchior (1975) have examined the possible involvement of serotonin in ethanol intake. Destruction of serotonergic nerves resulted in an increase in ethanol preference. Attempts to lower the cerebral levels of serotonin have generated generous quantities

of conflicting data (Deitrich, 1976). Effects on ethanol consumption varied depending on the conditions employed. In contrast, authors concur that the administration of the serotonin precursor, 5-hydroxytryptophan, significantly attenuated the imbibition of ethanol by rats (Geller, Purdy and Merritt, 1973; Zabik, Liao and Maickel, 1977).

Acute administration of ethanol to mice and rats produces a narcosis in which the animals lose their ability to right themselves from a supine position. Genetic factors operate to establish metabolic or sensitivity variations in the duration of ethanol narcosis (Belknap et al., 1972; Damjanovich and MacInnes, 1973; Heston et al., 1974; Erwin, Heston and McClearn, 1976; Malila, 1978). In addition, the actions of neurotransmitters seem to be associated with the production or maintenance of ethanol-induced narcosis. Originally, Rosenfeld (1960) demonstrated that the peripheral administration of neuroamines significantly prolonged the duration of the narcosis in mice. Many researchers have subsequently expanded that work. Inhibition of catecholamine synthesis through the use of alpha-methyl-p-tyrosine significantly lengthened the duration of ethanol-induced narcosis (Blum et al., 1972a). Decreases in the central levels of dopamine and noradrenaline accompanied the change. On the other hand, the administration of dopamine or L-DOPA, which served to increase the catecholamine levels, led also to increases in the duration of the loss of the righting reflex (Blum et al., 1973a; Messiha, Croy and Geller, 1974; Messiha, Morgan and Geller, 1975). Furthermore, inhibition of the central penetration of L-DOPA did not alter on narcosis duration (Messiha et al., 1974). These results

seemed to argue for the involvement of dopamine or one of its metabolites, perhaps at dopamine receptors (DiChiara et al., 1976). Those inhibitors of DBH that were able to penetrate the blood-brain barrier prolonged ethanol-induced narcosis in mice significantly (Hidaka et al., 1974). Catecholamines were also implicated in the studies that demonstrated that the adrenaline-induced increase in ethanol narcosis duration was blocked by pretreatment with phentolamine and potentiated by propranolol (Hanig, Prosky and O'Dell, 1971). Recently, results from other work with propranolol suggested that in addition to any effect the agent may have had on catecholamine receptors, propranolol may have exerted a depressant effect on neuronal membranes (Wimbish, Martz and Forney, 1977). Such an effect on the membranes could perhaps add to any similar membrane effects ethanol may exert.

Some narcosis studies have pointed to the serotonergic system as a possible participant in the central depressant activity of ethanol (Blum et al., 1973c, 1974a). Although treatment with serotonin significantly prolonged ethanol-induced narcosis, 5-hydroxytryptophan did not.

Narcosis tests were performed in which cholinergic agents were employed (Burnman and Erickson, 1969). Pretreatment with physostigmine significantly shortened the duration of ethanol-induced narcosis. Atropine had no apparent effect on the result observed with physostigmine and ethanol.

Investigations are currently being conducted into the involvement of other factors in ethanol narcosis prolongation. Thyrotropin-releasing hormone and a related compound significantly shortened the duration of the reflex loss (Breese et al., 1974; Prasad, Matsui and Peterkofsky, 1977). Hyberbaric oxygen was also effective in decreasing the narcosis duration (Alkana, Syapin and Noble, 1978).

In the ethanol-induced alterations in locomotor activity (Holtzman and Schneider, 1974) both genetic factors and neurotransmitter mechanisms have been considered. In mice strain differences in sensitivity to ethanol appeared in the form of variations in depression of locomotor activity (Randall et al., 1975). The authors suggested that interstrain neurochemical differences may have been involved in the response differences. In fact, alpha-methyl-p-tyrosine had previously been shown to suppress the locomotor stimulation produced by low doses of ethanol (Carlsson, Engel and Svennson, 1972). Treatment with L-DOPA reversed the suppression (Engel et al., 1974) whereas dopamine agonists mimicked the action of alpha-methyl-p-tyrosine (Carlsson et al., 1974). Alpha-adrenergic blockade antagonized the low-dose ethanol stimulation of locomotor activity but showed no effect on the high-dose depression. Beta-adrenergic blockade produced the reverse effect (Matchett and Erickson, 1977). These authors observed an enhancement of the ethanol-induced depression by dopaminergic receptor blockade. Agents resembling GABA decreased the locomotor stimulation produced by ethanol (Cott et al., 1976).

In human subjects, evidence of catecholamine involvement in ethanol-induced depression is meagre at present. Ethanol produced behavioural stimulation and euphoria that was significantly reduced by pretreatment with alpha-methyl-p-tyrosine (Ahlenius et al., 1973), and the administration of propranolol during acute intoxication has increased the central depressant effects of ethanol (Alkana et al., 1976).

Attempts to develop animal models of dependence on ethanol and tolerance to ethanol have been perfected to the point of enabling researchers to examine many of the parameters associated with these two phenomena (Ellis and Pick, 1973; Mello, 1976; Friedman and Lester, 1977; McMillan et al., 1977). Consequently, neurotransmitter receptors, their activation and possible role in dependence and tolerance (Collier, 1965) have been more easily scrutinized.

Mice exposed continually to ethanol vapour undergo withdrawal seizures after they are removed from the inhalation chambers (Goldstein and Pal, 1971; Goldstein, 1972; Griffiths, Littleton and Ortiz, 1973). The administration of alpha-methyl-p-tyrosine during a brief course of ethanol inhalation significantly increased the severity of the seizures (Blum and Wallace, 1974). Measurements of neurochemical concentrations in the brain in 3-day tests (Chopde, Brahmankar and Shripad, 1977) or 10-day tests (Griffiths, Littleton and Ortiz, 1974) showed that noradrenaline, dopamine and serotonin were increased and GABA was decreased during ethanol inhalation. Other than GABA levels, neuroamine concentrations returned to normal

during withdrawal prior to the time of maximal seizure scores. Furthermore, Goldstein (1973) observed that agents acting on the GABA system significantly modified the severity of the convulsions. Her work also told of aggravation of the seizures by agents that disrupted catecholaminergic function. Reserpine was more effective than alpha-methyl-p-tyrosine, phentolamine or propranolol. Lack of an effect was reported for serotonergic or cholinergic agents (Goldstein, 1973; Griffiths et al., 1974). Head twitches generated during ethanol withdrawal were reduced in number after treatment with dibutyryl cyclic AMP and with p-chlorophenylalanine (Collier, Hammond and Schneider, 1974, 1976).

The acute and chronic development of tolerance after ethanol administration has been studied in several animal models (Gibbins, Kalant and Le Blanc, 1968; Kalant, Le Blanc and Gibbins, 1971; Ritzmann and Tabakoff, 1976a; Tullis et al., 1977). Mice treated with 6-hydroxydopamine did not develop tolerance to the narcotic action of ethanol but did develop dependence, as demonstrated by the temperature changes experienced during withdrawal (Ritzmann and Tabakoff, 1976b). The administration of p-chlorophenylalanine prior to ethanol treatment accelerated the loss of tolerance but exerted no effect after tolerance had been established (Frankel et al., 1978). For an analysis of the concept of tolerance to centrally-acting agents the reader is referred to Le Blanc and Cappell (1977).

Simple cations have been observed to influence the behavioural responses to ethanol. Both clinically and in the laboratory lithium

reduced the preference for ethanol (Wren et al., 1974; Ho and Tsai, 1976). In rats lithium produced little change in ethanol withdrawal responses (Ho and Tsai, 1976). At higher doses of lithium the severity of the ethanol withdrawal responses was exacerbated. It appears that lithium may produce its effects by means of a mechanism that involves calcium ions. Lithium decreased the uptake of calcium into synaptosomes prepared from the brains of ethanol-consuming rats (Ross and Cardenas, 1978). Calcium ions, especially in the presence of ionophores, significantly increased the duration of ethanol-induced narcosis in mice (Erickson, Tyler and Harris, 1978). In contrast, after the administration of a calcium chelator the narcosis duration was markedly decreased.

h. Biogenic Amine Metabolites in the Actions of Ethanol

Ethanol consumption is known to divert the metabolism of catecholamines and serotonin from oxidative pathways to reductive pathways (Davis et al., 1967a, 1967b; Carter et al., 1970). Acetaldehyde was designated as the immediate culprit of the action. The requisite intermediates formed along either the oxidative or the reductive route are aldehydes generated through the action of MAO on the neuroamines. However, the aldehydes are so rapidly taken up and metabolized that they apparently exert little pharmacological activity (Renson, Weissbach and Udenfriend, 1964). On the other hand, the reduced metabolites have been observed to produce effects, especially on neuronal parameters. It was demonstrated that the reduced congeners of serotonin, which are alcohols in structure, produced a loss of the righting reflex in mice (Feldstein, Chang and

Kucharski, 1970). Furthermore, the serotonergic alcohols and the alcohol derived from dopamine, namely, 3,4-dihydroxyphenylethanol, significantly prolonged the narcosis induced by ethanol in mice (Blum *et al.*, 1973b; Feldstein, 1973). Any role that the reduced metabolites may have in the expression of the effects of ethanol apparently arises after acute or shortterm exposure to alcohol, as oxidative pathways for its removal become gradually more available upon chronic exposure to ethanol (Truitt, 1973). The author also suggested that the cross-tolerance observed between alcohol and barbiturates may involve some AldDH-mediated step, as was suggested by the results of subsequent experiments (Davis *et al.*, 1974).

Other aberrant metabolites of biogenic amines have been implicated in the expression of alcoholism. Acetaldehyde produced during the metabolism of ethanol has been hypothesized to condense with one or more of the endogenous amines to form an isoquinoline or beta-carboline. In turn, such condensates may act to produce some of the effects observed after ethanol intake (Cohen and Collins, 1970; Davis and Walsh, 1970; Walsh, 1973). Furthermore, acetaldehyde-induced enhancement of the formation of the morphine precursor tetrahydropapaveroline (THP), the condensate of dopamine and its aldehyde, presented a possible mechanism for associating alcohol and opiate depression or dependencies (Davis and Walsh, 1970). The furor directed towards the initial suggestions (Halushka and Hoffmann, 1970; Seevers, 1970) was succeeded by diverse research projects on the role of amine-aldehyde condensates in alcohol and opiate sequelae. Several reviews have been devoted at least in part to an examination

of the hypothesis (Caldwell and Sever, 1974; Rahwan, 1975; Cohen, 1976; Blum et al., 1978; Myers, 1978). The biological syntheses and actions of the relevant isoquinolines and beta-carbolines are presented earlier in this thesis. The effects that ethanol and opiates share with each other and with the condensation products will be discussed in the following paragraphs.

i. Effects Common to Ethanol, Opiates and Isoquinolines

Intracisternal injection of salsolinol, the tetrahydroisoquinoline (TIQ) formed from dopamine and acetaldehyde, generated a loss of the righting reflex in mice (Church, Fuller and Dudek, 1976). The authors reported that the ethanol-insensitive short-sleep strain of mice treated with salsolinol lost the reflex for a significantly shorter length of time than the ethanol-sensitive long-sleep strain of mice. At low doses of salsolinol the ethanol-sensitive strain demonstrated a greater increase in locomotor activity than the ethanol-insensitive strain.

Given in acute and chronic treatment regimens isoquinolines and beta-carbolines led to significant alterations in the pattern and amount of ethanol consumed by rats. In one report the acute peripheral administration of tryptoline, a nonsubstituted beta-carboline, significantly decreased voluntary ethanol consumption (Messiha, Larson and Geller, 1977). By far the most informative reports have emanated from the laboratory of Myers. Enhanced consumption of ethanol was observed in rats after the chronic intraventricular infusion of THP, salsolinol, a methoxyl derivative

of the noradrenaline-formaldehyde condensate of tryptoline (Melchior and Myers, 1977; Myers and Melchior, 1977a, 1977b; Myers, 1978). The noradrenaline-formaldehyde TIQ and 3-carboxysalsolinol produced smaller increases in the amount of alcohol consumed. It is especially noteworthy that the rats usually exhibiting an aversion towards ethanol were encouraged to consume vast quantities of the drug even as long as 6 months after the infusion of THP (Myers, 1978). Signs of ethanol intoxication and withdrawal were evident in many of the rats during the treatment programmes (Myers and Oblinger, 1977).

In mice TIQs effectively altered the severity of seizures attendant upon ethanol withdrawal. Intraventricular injections of norsalsolinol or salsolinol significantly intensified the seizures (Blum et al., 1976b, 1978). Apparently, the S(−)-form of salsolinol was the isomer responsible for the exacerbation of the withdrawal response observed with salsolinol.

Opiate-like effects have been reported for some TIQs. Salsolinol behaved with agonistic and antagonistic activities on opiate receptors in the field-stimulated preparation of guinea-pig ileum (Hamilton et al., 1976). Given intraventricularly, salsolinol enhanced the analgesic effect of morphine on the murine tail-clip test and interfered with antagonism of the response by naltrexone (Blum et al., 1976b). The stereospecific binding of tritiated naloxone to homogenates prepared from rat brain was weakly antagonized by THP, salsolinol and protoberberine alkaloids (Tampier, Alpers and

Davis, 1977). In a clinical test a dimethoxylated TIQ attenuated the withdrawal symptoms and signs in morphine addicts but to a much lesser degree than did codeine (Fraser et al., 1961). The agent was twice as potent as codeine in the clinical analgesia tests. Other isoquinoline congeners also have been shown to exert analgesic effects (Szegi et al., 1959).

Comparisons of the neural effects of alcohol and opiates given acutely or chronically have been discussed recently (Eidelberg, 1975; Green and Jaffe, 1977). In another report the common neurochemical and behavioural characteristics of the two centrally acting agents were considered in terms of a possible connection via TIQ actions (Blum, Hamilton and Wallace, 1977b). Of great importance to the argument are the findings of Ross, who examined the depletion of calcium from discrete areas of rat brain. In his initial study he observed that morphine, ethanol and salsolinol induced a calcium depletion that was prevented by pretreatment with naloxone (Ross et al., 1974). Calcium depletion caused by reserpine or pentobarbital was not affected by the prior administration of naloxone. In subsequent investigations on calcium levels morphine and ethanol displayed a cross-tolerance that probably involved protein synthesis (Ross, 1975, 1976). In addition, the morphine-induced reduction of the binding of calcium to synaptosomal membranes (Ross, Lynn and Cardenas, 1976) resembled the salsolinol-induced reduction of binding in its attenuation by naloxone (Ross, 1978). Furthermore, calcium has been implicated in the acute depressant activity of ethanol (Erickson, 1978) and opiates (Kakuanaga, Kaneto and Hano, 1966).

Other neurochemical changes and behavioural responses common to the action of ethanol and opiates have been reported. In rodent brains the turnover or levels of noradrenaline and dopamine were significantly increased by chronic administration of ethanol (Hunt and Majchrowicz, 1974) or morphine (Sloan et al., 1963). Acetaldehyde and morphine were observed to increase the concentration of acetylcholine in rodent brains (Berry and Stolz, 1956; Richter and Golstein, 1970). Also, the activity of adenylate cyclase in central sites was markedly enhanced by chronic ethanol or morphine treatment regimens (Bonnet, 1975; Kuriyama, 1977). After withdrawal from either agent adenylate cyclase activity was again increased (Blasig, Herz and Gramsch, 1975; Mehta and Johnson, 1975).

By altering neurochemical factors the actions of ethanol and opiates have been modified. For instance, the inhibition of central protein synthesis in the presence of cycloheximide significantly inhibited the development of tolerance to ethanol and morphine (Loh, Shen and Way, 1969; Le Blanc, Metsunaga and Kalant, 1976). The administration of calcium was able to block alcohol and opiate withdrawal hyperactivity responses (Sangvi and Gershon, 1976; Hamilton, Blum and Hirst, 1977). In many laboratories it was observed that treatment with catecholaminergic or serotonergic agents significantly altered the effects produced by opiates (Cheney and Goldstein, 1971; Ho et al., 1972; Buxbaum, Yarbrough and Carter, 1973; Sewell and Spencer, 1974, 1975; Iwamoto, Ho and Way, 1976; Ary and Lomax, 1977; Ferri, Reina and Santagostino, 1977). For a summary of neuroamine involvement in the actions of ethanol, the

reader is referred to previous sections.

Finally, ethanol and opiates were observed to alter the responses produced by the opposite agent. For instance, morphine significantly decreased ethanol consumption by hamsters, whereas the narcotic antagonist naltrexone increased the intake of ethanol (Ross *et al.*, 1976). Similarly, in rats morphine led to a suppression of ethanol consumption (Sinclair, Adkins and Walker, 1973). Low doses of naltrexone or naloxone produced an enhancement (Ho, Chen and Morrison, 1977). In other tests signs of drug dependence were altered. Chronic ethanol administration to rats coincident with chronic exposure to morphine significantly attenuated the severity of the naloxone-induced withdrawal from the opiate (Jones and Spratto, 1977). Furthermore, naloxone significantly reduced the severity of ethanol withdrawal seizures witnessed in mice (Blum *et al.*, 1977a).

E. Pharmacology of Acetaldehyde

Investigations into the relevance of acetaldehyde as a contributor to the responses observed after alcohol intake have been conducted in many laboratories. Whether acetaldehyde acts alone or as a component of some other reactant has been posed and examined in great detail (Rahwan, 1975; Truitt and Walsh, 1971).

In 1945, acetaldehyde was reported to behave as an anaesthetic and to stimulate sympathetically-innervated tissue (Koppanyi, 1945). Between that time and the present many studies have further

characterized its sympathetic actions. Increases in blood pressure were observed (Akabane et al., 1964), which were superceded at high doses by a depressor effect (Romano, Meyers and Anderson, 1954). Acceleration of heart rate was effected in humans in correlation with the blood acetaldehyde levels achieved after ethanol consumption (Zeiner, Paredes and Christensen, 1978). In dogs many haemodynamic properties were studied. In addition to the elevation in blood pressure and the positive chronotropic effect of acetaldehyde, the agent succeeded in dilating coronary vessels and increasing collateral blood flow (Bandow, Afonso and Rowe, 1977). Beta-receptor blockade by propranolol only slightly reduced the haemodynamic effects of acetaldehyde. Furthermore, it has been observed that acetaldehyde does not significantly alter the level of noradrenaline found in the heart (Svennson and Waldeck, 1973). However, an acetaldehyde-induced secretion of noradrenaline and adrenaline from the adrenal glands was reported (Akabane et al., 1965; Schneider, 1974), a secretion probably implicated in the pressor response to the aldehyde (Akabane et al., 1964).

Following acetaldehyde administration the alteration of neuroamine concentrations in brain has been recorded. Acute or chronic treatment with acetaldehyde significantly increased the levels of noradrenaline, dopamine and serotonin in mouse brain (Svennson and Waldeck, 1973; Ortiz et al., 1974). However, decreases in the brain concentration of noradrenaline was reported either for larger intraperitoneal doses or for intracisternally administered doses of acetaldehyde (Duritz and Truitt, 1966; Thadani and Truitt, 1977).

Other parameters of neuroamine existence have also been altered by acetaldehyde. In particular, the rates of metabolism of noradrenaline, dopamine and serotonin were changed. According to various reports, acetaldehyde acted as a competitive inhibitor of brain and liver AldDH (Lahti and Majchrowicz, 1967; Walsh, Davis and Yamanaka, 1970), which, in addition to its function in acetaldehyde metabolism, is responsible for converting the MAO-produced aldehydes of the neuroamines to their respective acids. One possible event involved in changing the metabolism of brain amines, especially serotonin, may be the acetaldehyde-induced increase in binding of neuroamine-derived aldehydes to brain tissue (Tabakoff, Ungar and Alivisatos, 1972). Furthermore, abnormal formation of the neuroamine-derived aldehydes has been observed as a consequence of MAO inhibition in brain and liver preparations incubated with acetaldehyde (Towne, 1964).

Acetylcholine was increased in quantity in the brains of rats in minutes after acetaldehyde administration (Berry and Stotz, 1956). But, ultimately the brain level of acetylcholine was noticeably decreased (Rawat, 1974).

Acetaldehyde was shown to produce a dose-dependent inhibition of sodium-, potassium- and magnesium-activated ATPase in rat brain synaptosomes (Tabakoff, 1974). In rat brain mitochondrial preparations acetaldehyde inhibited pyruvate oxidation, but no effect on the phosphorylation coupled to the oxidations was observed (Kiessling, 1962).

Behavioural responses to acetaldehyde have been reported by many authors. Mice demonstrated behavioural changes suggestive of central depression. The chronic inhalation of acetaldehyde vapour produced a reduction in the amount of spontaneous locomotor activity displayed (Ortiz et al., 1974). Fine and gross movements were distorted in the presence of acetaldehyde, a response culminating in immobility at higher doses (Holtzman and Schneider, 1974). Similar withdrawal syndromes were apparent upon the discontinuation of either chronic alcohol or chronic acetaldehyde administration (Griffiths et al., 1974b). However, that syndrome arising from the aldehyde was greater in intensity and shorter in duration.

Signs of acetaldehyde overdose were most clearly expressed as depressed cardiovascular and respiratory responses. The rate and rhythm of the ventricles and the depth of respiration were adversely affected (Koppanyi, 1945).

III MATERIALS

The tetrahydroisoquinolines used in the experiments were synthesized in this laboratory. The radioactive and radioneutral forms of 3-carboxysalsolinol were prepared according to modified versions of the method of Brossi et al. (1972) as described in Methods. Salsolinol and dimethoxysalsolinol were synthesized by Dr. M. Hirst.

Listed below are the chemicals that were employed:

1) Acacia

   British Drug Houses Ltd., Poole, England

   Lot number 908662

2) Alcohol, Anhydrous (ethanol)

   Mol. Wt. 46.1

   Fisher Scientific Company (Canada), Toronto, Ont.

3) Benserazide Hydrochloride (R04-4602/1)

   Mol. Wt. 293.3

   Kindly supplied by Dr. W. E. Scott of Hoffmann-La Roche Inc., Nutley, N. J.

   Lot number A395212

4) Carbidopa (MK 486)

Mol. Wt. 244.3

Kindly supplied by Dr. C. C. Porter of Merck, Sharp and Dohme Research Laboratory, West Point, Pa.

Lot number V-1-031-4

5) Cation Exchange Resin, Analytical Grade 50W-X4

Bio-rad Laboratories, Richmond, Ca.

Lot number 14351

6) DL-3,4-Dihydroxyphenyl(1-^{14}C)alanine ((^{14}C-COOH)-DOPA)

Mol. Wt. 199

Amersham Corporation, Oakville, Ont.

Batch 11, specific activity 53 mCi/mmol (See Methods for the preparation of the substance for experimental use.)

7) Dopamine Hydrochloride

Mol. Wt. 189.6

Sigma Chemical Company, St. Louis, Mo.

Lot number 90C-1660

8) Haloperidol (Haldol)

Mol. Wt. 375.9

McNeil Laboratories (Canada), Don Mills, Ont.

Lot number not recorded

9) Hexobarbital

Mol. Wt. 236.3

Street Chemicals and Company, Montreal, P.Q.

Lot number 079877 (Siegfried)

10) Hydrochloric Acid, Reagent Grade

Mol. Wt. 36.5

Fisher Scientific Company (Canada), Toronto, Ont.

Lot number omitted from label

11) Iodine Crystals, Analytical Grade

At. Wt. 126.91

Mallinckrodt Chemical Works, Montreal, P.Q.

Lot number 88449

12) Levodopa (L-dihydroxyphenylalanine, L-DOPA)

Mol. Wt. 197.2

Sigma Chemical Company, St. Louis, Mo.

Lot number 66C-0328

13) 1-Methyl-3-carboxy-6,7-dihydroxy-1,2,3,4-tetrahydroisoquinoline

(3-carboxysalsolinol)

Mol. Wt. 223.2

M. Pt. 272 -275 C.

Synthesized by the author (See Methods)

14) 1-Methyl-(^{14}-C-COOH)-3-carboxy-6,7-dihydroxy-1,2,3,4-tetrahydroisoquinoline ((^{14}C-COOH)-3-carboxysalsolinol)

Mol. Wt. 225

M. Pt. 272 - 275 C.

Synthesized by the author (See Methods)

15) 1-Methyl-6,7-dihydroxy-1,2,3,4-tetrahydroisoquinoline Hydrochloride (salsolinol)

Mol. Wt. 215.6

Synthesized by Dr. M. Hirst, Dept. of Pharmacology, University of Western Ontario, London, Ont.

16) 1-Methyl-6,7-dimethoxy-1,2,3,4-tetrahydroisoquinoline Hydrochloride (dimethoxysalsolinol)

Mol. Wt. 242.6

Synthesized by Dr. M. Hirst, Dept. of Pharmacology, University of Western Ontario, London, Ont.

17) Morphine Hydrochloride

Mol. Wt. 321.8

Merck and Company Ltd., Montreal, P.Q.

Lot number 22956

18) Naloxone

Mol. Wt. 327.4

Kindly supplied by Endo Laboratories Inc., Garden City, N. Y.

Lot number 9054Z-1

19) NCS™ Solubilizer

Amersham Corporation, Oakville, Ont.

Lot number 742

20) Pargyline Hydrochloride (Eutonyl)

Mol. Wt. 195.7

Abbott Laboratories, Montreal, P.Q.

Lot number 762-7511

21) PCS™ Solubilizer

Amersham Corporation, Oakville, Ont.

Lot number 06698

22) Potassium Carbonate, Analytical Grade

Mol. Wt. 138.21

British Drug Houses Ltd., Poole, England

Lot number 48353

23) Potassium Iodide, Reagent Grade

Mol. Wt. 166.02

Merck and Company Ltd., Montreal, P.Q.

Lot number 352214

24) Potassium Phosphate Monobasic, A.C.S. Certified

Mol. Wt. 136.09

Fisher Scientific Company (Canada), Toronto, Ont.

Lot number 742804

25) Pyrazole

Mol. Wt. 68.1

Aldrich Chemical Company, Inc., Milwaukee, Wis.

Lot number 030871

26) Pyridoxal-5'-phosphate

Mol. Wt. 247.2

Sigma Chemical Company, St. Louis, Mo.

Lot number 26C-0330

27) Sodium Acetate, Reagent Grade

Mol. Wt. 82.03

J. T. Baker Chemical Company, Phillipsburg, N. J.

Lot number 33182

28) Sodium Chloride, A.C.S. Certified

Mol. Wt. 58.44

Fisher Scientific Company (Canada), Toronto, Ont.

Lot number 762578

29) Sodium Sulfite Anhydrous, Reagent Grade

Mol. Wt. 126.05

Merck and Company Ltd., Montreal, P.Q.

Lot number 3617[illegible]0

30) Tetraethylthiuram Disulfide (disulfiram)

Mol. Wt. 296.5

Aldrich Chemical Company, Inc., Milwaukee, Wis.

Lot number 102327

IV METHODS

A. Chemical Preparations

1. Formation of 3-Carboxysalsolinol

Radioneutral and radiolabelled 3-carboxysalsolinol were produced according to modified versions of the technique reported by Brossi et al. (1972). Basically, the products were formed from condensation of DOPA with acetaldehyde. The authors reported that the method generates 95% of the product in the "cis" isomeric form.

In the initial step in the formation of radioneutral 3-carboxysalsolinol, L-DOPA (2.17 g) was mixed with sulfuric acid (21 ml, 0.5 N) and acetaldehyde (5.0 ml). While being gassed with nitrogen the mixture was stirred for 2 hr at 50 C and then for 24 hr at room temperature. The resulting crystals were filtered, washed with double-distilled de-ionized water and dried. The yield of crystals was 0.7 g. The filtrate was neutralized with 20% (w/v) sodium hydroxide, stored at 4 C for 24 hr and filtered. Crystals obtained from the filtrate were added to those originally produced. Boiling water was gently poured over the crystals. Filtration and drying yielded 1.2 g of crystals, mp 273-275 C. Crystals produced by Brossi et al. (1972) displayed a mp 280-281 C. The crystals were stored at 0 C. Thin-layer chromatography of the crystals was conducted on silica plates (Eastman) using a 20:20:60 combination of glacial acetic acid, isopropyl alcohol and chloroform. The plates were sprayed with a solution of potassium permanganate for

chromatographic development. The chromatograms indicated one spot, which matched that of authentic 3-carboxysalsolinol. No spot corresponding to that of L-DOPA was apparent.

The synthesis of (^{14}C-COOH)-3-carboxysalsolinol was conducted according to a microsynthetic version of the technique just described. Radioneutral L-DOPA (25 mg) was added to 50 μCi of DL-DOPA (5 mg). Sulfuric acid (0.3 ml, 0.5 N) and acetaldehyde (0.2 ml) were then added. The mixture was stirred under nitrogen at room temperature. Additional acetaldehyde (0.1 ml) was added at 20 min and at 30 min. The total mixing time was 60 min. Crystals that formed were then filtered. Boiling, double-distilled de-ionized water was added drop-wise to the crystals and filtered through them. The crystals were dried to give 10.84 mg. Acetaldehyde (0.2 ml) and radioneutral 3-carboxysalsolinol (138 mg) were added to the filtrate. Ethanol (1 ml) was then added to form a homogeneous solution. Diethyl ether was then slowly introduced down the side of the vessel. Crystalline materials began to separate out. After 24 hr the crystals were filtered from the solution. The crystals (125.23 mg) were dried, stored at 0 C overnight and subjected to thin-layer chromatography as described previously. Crystal samples (3.09 mg) of each crop were dissolved in 1.875 ml of acidic saline (pH 4). A calibrated 5-μl pipette was used to determine the radioactivity of the samples. The specific activities determined were as follows: crop 1 crystals, 2.19×10^6 dpm/mg; crop 2 crystals, 8.02×10^4 dpm/mg. The yield of crop 1 was calculated to be 50.5%.

2. Preparation of (1-^{14}C)-DOPA

Radioneutral L-DOPA (25 mg) was added to 50 µCi of (1-^{14}C)-DL-DOPA (5.0 mg). The resulting mixture was dissolved in acidic saline (15.6 ml, pH 4). The specific activity of the solution was 4.11×10^7 dpm/mg.

B. Behavioural Experiments

1. Ethanol-induced Narcosis

Male, Swiss-Webster albino mice weighing between 24 and 32 g were the subjects chosen. Prior to testing, the mice were housed in colonies in the presence of a 12-hr light/12-hr dark cycle for at least 3 days. They were permitted access to water and laboratory chow *ad lib*, until the morning of the test. The protocol followed was the narcosis duration technique developed by Kakihana *et al.* (1966). All experiments were conducted at an ambient temperature of 22.0 ± 1.0 C. The tests were performed always during the late morning. Following drug administration the mice were housed in individual clear plastic cages, and the time at which each subject lost the reflex to right himself from a supine position was recorded. The time at which the mouse had righted himself twice within an interval of 30 sec was selected as the end of the narcosis duration. A value of 150 min was chosen arbitrarily as the limit of the narcosis duration for any subject that had not regained the righting reflex by the end of that time period. At least 10 subjects were assigned to the treatment groups by a random number placement.

Initially, all agents were examined individually for the ability to produce narcosis. Ethanol was prepared as a 25% (v/v) concentration in physiological saline (0.9% NaCl) and was administered in a dose of 43.5, 65, 87, 109, 130 or 174 mmoles/Kg body wt. The injected volume of solution was 20 µl/g body wt, and was delivered intraperitoneally via a disposable 26-gauge hypodermic needle. Each of L-DOPA, dopamine, 3-carboxysalsolinol, salsolinol and dimethoxysalsolinol was prepared in physiological saline and was administered intraperitoneally (5 µl/g body wt) as just described. The five agents were administered in a concentration of 60 µmoles/Kg body wt. Except for dimethoxysalsolinol, the agents were given also by the intravenous route (2 µl/g body wt) by way of a 30-gauge hypodermic needle and in a dose of 60 µmoles/Kg body wt. In order to aid dissolution of the amino acid compounds, L-DOPA and 3-carboxysalsolinol, the saline solutions of these agents were brought to pH 4 with hydrochloric acid (5.0 N).

In some experiments the administration of other drugs immediately preceded the injection of ethanol, 87 mmoles/Kg (4 g/Kg). The agents, which were given intraperitoneally in a volume of 5 µl/g body wt, were as follows: L-DOPA (60, 250 or 1000 µmoles/Kg), dopamine (60 umoles/Kg), 3-carboxysalsolinol (7.5, 10, 15, 30, 60 or 120 µmoles/Kg), salsolinol (60, 460, 920, 1380 or 1840 µmoles/Kg) and dimethoxysalsolinol (60, 410 or 820 µmoles/Kg).

A variety of pretreatment regimens was incorporated into the testing protocol as a means of either inhibiting particular enzymes

or blocking a certain species of receptors. The chemical structures of the inhibitors are illustrated in Appendix 1. The alcohol dehydrogenase inhibitor pyrazole (0.5 or 1 mmole/Kg) was prepared in saline and was administered intraperitoneally in a volume of 2 μl/g body wt 30 min before the injection of ethanol. In addition, pyrazole (0.5 mmole/Kg) was employed in tests in which the administration of ethanol was immediately preceded by that of one of the following agents: L-DOPA (1000 μmoles/Kg), dopamine (60 μmoles/Kg), 3-carboxysalsolinol (60 μmoles/Kg) or salsolinol (60, 920 or 1380 μmoles/Kg). An inhibitor of both aldehyde dehydrogenase and dopamine-beta-hydroxylase, disulfiram (0.25, 0.50 or 1.01 mmoles/Kg), was prepared as a suspension in 5% gum acacia warmed to 37 C. The disulfiram suspension was administered intraperitoneally in a volume of 10 μl/g body wt 24 hr before the ethanol (87 mmoles/Kg) injection. In the subsequent combination tests pretreatment with disulfiram (0.25 mmoles/Kg) was followed by treatment with ethanol (87 mmoles/Kg) and one of the following drugs: L-DOPA (60 or 1000 μmoles/Kg), dopamine (60 μmoles/Kg), 3-carboxysalsolinol (60 μmoles/Kg) or salsolinol (60 or 920 μmoles/Kg). The monoamine oxidase inhibitor pargyline (51, 102 or 510 μmoles/Kg) was prepared in saline and injected in a volume of 10 μl/g body wt 4 hr before ethanol (87 mmoles/Kg) was administered. Also, the administration of pargyline (51 μmoles/Kg) was followed by that of ethanol (87 mmoles/Kg) and one of L-DOPA, dopamine, 3-carboxysalsolinol or salsolinol at a dose of 60 μmoles/Kg.

Experiments were conducted in which an inhibitor of either L-aromatic-amino-acid decarboxylase or dopamine receptors was employed.

In order to inhibit decarboxylase in peripheral tissues carbidopa (102 μmoles/Kg) was delivered at 37 C as a suspension in 5% gum acacia in saline. The suspension was administered in a volume of 10 μl/g body wt via a gastric tube (Portex size 3 flexible nylon tubing on a curved 20-gauge needle) 1 hr before the ethanol (87 mmoles/Kg) treatment. Tests were performed incorporating carbidopa pretreatment with the subsequent administration of ethanol (87 mmoles/Kg) and L-DOPA (60, 250 or 1000 μmoles/Kg) or 3-carboxysalsolinol (7.5 or 60 μmoles/Kg). As a means of inhibiting the decarboxylase in central and peripheral locations benserazide hydrochloride (1.7 mmoles/Kg) was prepared in saline and administered intraperitoneally in a volume of 10 μl/g body wt 1 hr before ethanol (87 mmoles/Kg) treatment. In the ensuing tests pretreatment with benserazide was followed by the injection of ethanol (87 mmoles/Kg) and either L-DOPA (250 or 1000 μmoles/Kg) or 3-carboxysalsolinol (7.5, 30 or 60 μmoles/Kg). The final pretreatment test involved the intraperitoneal delivery of the dopamine receptor blocker haloperidol (75, 150 or 300 nmoles/Kg) in a volume of 5 ul/g body wt 3 hr before ethanol (87 mmoles/Kg) administration.

Saline cotreatment and saline and acacia pretreatment control tests were performed. Either saline or acidic saline was delivered in a volume of 5 μl/g body wt immediately before the administration of ethanol (87 mmoles/Kg). Saline in a volume of 10 μl/g body wt was administered 30 min before ethanol (87 mmoles/Kg) treatment. The acacia solution, in a volume of 10 μl/g body wt, was delivered via a gastric tube 1 hr or 24 hr before the injection of ethanol (87 mmoles/Kg).

2. Hexobarbital-induced Narcosis

Swiss-Webster albino male mice, 22 to 28 g, were the subjects in the hexobarbital sleeping time experiments. The testing method used was based on that of Kakihana *et al.* (1966) under the conditions described previously. Hexobarbital was prepared in physiological saline and was administered in a dose of 0.39 mmoles/Kg (100 mg/Kg). The solution of hexobarbital was delivered in a volume of 20 μl/g body wt by the intraperitoneal route, by way of a disposable 1-ml tuberculin syringe with a disposable 26-gauge hypodermic needle. Both L-DOPA and 3-carboxysalsolinol were prepared in the form of acidic saline solutions and were administered intraperitoneally (5 μl/g body wt). Experiments were conducted in which the administration of hexobarbital was immediately preceded by an injection of either L-DOPA (38, 190 or 100 μmoles/Kg) or 3-carboxysalsolinol (38 or 190 μmoles/Kg). In some tests the mice were pretreated with carbidopa, an inhibitor of peripheral L-aromatic amino-acid decarboxylase. The subjects received the carbidopa (102 μmoles/Kg) orally as a suspension in warmed 5% gum acacia in saline, delivered in a volume of 10 μl/g body wt. One hr after the carbidopa pretreatment, hexobarbital and either L-DOPA (7.5 or 250 μmoles/Kg) or 3-carboxysalsolinol (3.8 or 7.5 μmoles/Kg) were injected.

Control experiments were conducted incorporating acidic saline cotreatment and acacia pretreatment. Acidic saline was injected in a volume of 5 μl/g body wt just prior to hexobarbital administration. One hr before an injection of hexobarbital, a solution of 5% acacia in saline was administered orally in a volume of 10 μl/g body wt.

3. Tail-clip Analgesia

Male, Swiss-Webster albino mice, ranging in weight from 20 to 25 g, were the subjects in all of the analgesia tests. The mice were housed in colony cages under a 12-hr light/12-hr dark cycle for at least 3 days prior to testing. They were permitted ad lib. access to water and to laboratory chow until the morning of the experiment. All tests were performed during late morning or early afternoon. Analgesia was measured according to the modification by Brands et al (1976) of Haffner's tail-clip method (Haffner, 1929). The clip used in the tests (Medicon) had arms 2 cm in length, each arm being encased in polyethylene tubing to prevent tissue damage. Thirty min after drug injection the clip was placed at the base of the tail, and the latency to respond to the clip by biting it was recorded. A value of 10 sec was assigned if the mouse had not responded to the clip by that time. Each treatment group consisted of 30 mice assigned randomly.

3-Carboxysalsolinol (2.2, 4.4, 11, 44, 110, 220, 440 and 880 μmoles/Kg) and L-DOPA (4.4, 44 or 220 μmoles/Kg) were prepared in physiological saline adjusted to pH 4 to aid dissolution of the drugs. Morphine (11, 16.5, 22.0 μmoles/Kg) and naloxone (3.0 μmoles/Kg) were dissolved in normal physiological saline. The drugs were administered intraperitoneally by means of a disposable 1-ml tuberculin syringe with a disposable 26-gauge, hypodermic needle. All drugs were injected in a volume of 5 μl/g body wt, except for 3-carboxysalsolinol (880 μmoles/Kg) and carbidopa (102 μmoles/Kg), which were

delivered in solutions prepared as 10 μl/g body wt. Carbidopa (102 μmoles/Kg) was administered as a suspension in warmed 5% gum acacia in saline, by means of a gastric tube, 1 hr prior to the administration of other agents.

The amino acid compounds, 3-carboxysalsolinol and L-DOPA, were compared for their ability to produce analgesia. In these tests the following treatments were employed: 3-carboxysalsolinol (2.2, 4.4, 44, 110, 220, 440 or 880 μmoles/Kg), carbidopa pretreatment (102 μmoles/Kg) and 3-carboxysalsolinol (2.2, 4.4, 11 or 44 μmoles/Kg), L-DOPA (4.4, 44 or 220 μmoles/Kg), and carbidopa pretreatment (102 μmoles/Kg) and L-DOPA (4.4 μmoles/Kg). 3-Carboxysalsolinol was initially screened for the intensity and duration of its analgesic activity. In control tests acidic saline in a volume of 5 μl/g body wt was administered intraperitoneally 1 hr after carbidopa (102 μmoles/Kg) pretreatment. Another control test involved pretreatment with acacia (10 μl/g body wt) followed by 3-carboxysalsolinol (4.4 μmoles/Kg) 1 hr later.

Morphine-induced analgesia and the alteration of the responses by concomitant treatment with other agents was also examined. An injection of morphine (11, 16.5 or 22 μ-moles/Kg) was immediately preceded by acidic saline or by either 3-carboxysalsolinol or L-DOPA in a concentration of 44 or 220 μmoles/Kg. Subsequently, tests were performed in which carbidopa (102 μmoles/Kg) pretreatment was followed by the administration of morphine (11 μmoles/Kg) and either 3-carboxysalsolinol or L-DOPA (4.4 μmoles/Kg) or acidic saline. In

one test, morphine treatment followed 1 hr after the administration of acacia. In some experiments the narcotic antagonist naloxone (3.0 μmoles/Kg) was prepared for delivery in saline (5 μl/g body wt) and administered immediately prior to the injection of 3-carboxysalsolinol, L-DOPA, morphine or combinations of agents with or without carbidopa (102 μmoles/Kg) pretreatment. The tests in which the doses of morphine and/or the amino acids resulted in the greatest percentage of mice analgesic were those that were repeated in the presence of naloxone (3.0 μmoles/Kg).

4. Spontaneous Locomotor Activity

Male, Swiss-Webster albino mice, 22 to 28 g at the time of testing, were used in the spontaneous locomotor activity tests. They were maintained on laboratory chow and water *ad lib*. For 5 days prior to testing, as well as during the course of the experiment, the subjects were housed under a 12-hr light/12-hr dark cycle.

Activity was measured by a Varimex Activity Meter (Columbus Instruments) having two horizontal sensors, each connected to a digital counter with a print-out. The meter was set up to record electromagnetically movement across the floor of each of two black-walled plexiglas boxes, which had ventilated black lids. Five mice were randomly assigned to each box, a drug-injected group in one box and a saline-injected group in the other box . The testing was conducted in a darkened room and always between 8:00 PM and 10:00 PM, the first 2 hr of the dark cycle.

In order to obtain baseline levels of activity, non-injected mice were tested for activity on two or more consecutive nights until a stable baseline activity was achieved. Subjects demonstrating stable baseline activity were injected, intraperitoneally, at 8:00 PM on the ensuing evening with either acidic saline or 3-carboxysalsolinol (38, 100 or 190 μmoles/Kg) dissolved in acidic saline. The delivery of either solution was by means of a 1-ml tuberculin syringe fitted with a 26-gauge disposable needle. The volume injected was 15 μl/g body wt. At least four tests were conducted with each dose of the drug.

The mice were tested for spontaneous locomotor activity for a duration of 2 hr immediately following the injection of either the isoquinoline or saline. For each group of five mice the percentage change from baseline activity was calculated for the responses recorded during five 6-min intervals. The ratio of the number of movements recorded after injection to the number of movements recorded as baseline activity was calculated.

5. Statistical Analysis of Behavioural Tests

The data obtained in the narcosis tests and in the spontaneous locomotor activity tests were analyzed with the aid of a Wang 600 Series calculator. Programme number 1014-2-ST1 was employed to determine the mean, variance and standard error of the mean for ungrouped data. To perform Student's two-tailed t-test for significant difference in the mean for the unpaired data, programme number 1010-2-ST2 was used. The results of the analgesia tests were analyzed by the method outlined by Goldstein (1964) for estimating the difference

between sample proportions. In all experiments the results were considered to be significant at $P < 0.05$.

C. Decarboxylation of DOPA and 3-Carboxysalsolinol

1. Alteration of Dopamine and Carbon Dioxide Formation *In Vitro*

a. Introduction

Anaerobic production of dopamine from L-DOPA by a guinea-pig kidney homogenate was determined in the presence and in the absence of 3-carboxysalsolinol. First of all, in order to perform the assays, incubation, extraction and fluorometric techniques were modified from published methods. Descriptions of the techniques employed will be found in the following sections.

b. Preparation of the Guinea-pig Kidney Homogenate

The method reported by Schales and Schales (1949) formed the basis of the decarboxylase enzyme preparation technique. Each of seven male guinea-pigs weighing between 300 and 350 g, were stunned by a blow on the head, exsanguinated, and the abdominal cavity was exposed. The kidneys were rapidly removed, weighed and placed in two 10-ml beakers, each of which contained chilled double-distilled, de-ionized water (6.0 ml). After 4 min the beakers containing the kidneys were put into a refrigerator (4 C) for 1 hr. At the end of that time the water was drained, and each kidney was added to chilled potassium phosphate buffer (3.0 ml, 0.5 M), pH 6.9, in a 12-ml polypropylene centrifuge tube. The tubes were placed in an ice-bath,

and then the kidneys were homogenized by a Brinkman Polytron homogenizer operated at position number 6 for 13 sec. Phosphate buffer was added to the concentrated kidney solution in a quantity that would result in a 10% (w/v) homogenate of the kidneys. The mixture was then centrifuged at 12,000 g for 10 min. The supernatant was decanted and subsequently frozen at -70 C in volumes of 1.2 ml in plastic lidded Eppendorf centrifuge tubes or else used immediately.

c. Incubation of the Kidney Supernatant Fraction

The incubation technique was based on the method developed by Thomas (1969) for a study on decarboxylase activity in the housefly and on that developed by Lovenberg et al. (1962). The following solutions were pipetted into the main compartment of a Warburg flask: potassium phosphate buffer (1.0 ml, 0.5 M), pH 6.9; of pyridoxal-5'-phosphate (0.1 ml, 1.7 mM); 1.0 ml of the kidney supernatant fraction. A solution of L-DOPA (5 mM) was pipetted (0.2 ml) into the sidearm. If 3-carboxysalsolinol was used in an experiment, 0.5 ml of a solution of the isoquinoline was pipetted into the sidearm, and the amount of phosphate buffer in the main compartment was reduced by the same volume. This yielded a final concentration of 100 and 200 μM for the isoquinoline. "Tissue blanks" and reagent blanks" were also prepared.

The prepared flasks were placed on the gassing sidearms of manometers and lowered into the water reservoir (37 C) of a Gilson respirometer. The fluid in the columns of the manometers was raised to the 100 mm mark just prior to the nitrogen being introduced into

the system. With the flasks being shaken and the water being stirred, the system was gassed with nitrogen for 10 min. The manometer fluid was then lowered to below the 0 mm line, and the nitrogen was turned off. The sidearms were rotated to the closed position, and the manometers were placed on the bath for 10 min more. After that time had elapsed, the fluid was raised to the 10 mm line in the right column of the manometer, the reading in the left column was recorded, a finger was placed over the open top of the left column and the sidearm contents were tipped into the main compartment. Each flask was incubated for 10 min, after which time the fluid in the right column was raised to the 10 mm line, the fluid level in the left column was recorded, and the flask was removed from the bath. A volume (1 ml) of the reaction mixture was extracted from the flask and placed in a glass test tube in a boiling water bath for 1 min. To the boiled sample were added double-distilled, de-ionized water (0.2 ml) and HCl (0.8 ml, 0.2 N) Samples were obtained from each of 9 flasks incubated according to any given set of incubation conditions.

d. Extraction of Dopamine from the Incubate

The method used to extract dopamine was that described by Bertler and Rosengren (1959), with modifications. Extensive preliminary tests were conducted with resin columns to determine the conditions necessary to separate L-DOPA and 3-carboxysalsolinol from dopamine and salsolinol.

A plug of cotton batting was placed above the main construction of a 23 cm-long Pasteur pipette to a depth of 1.5 cm. The resin used

in the tests was Dowex 50W-X4 that had been slurried in 5.0 N hydrochloric acid for 20 min. Resin was placed to a depth of 2.5 cm on top of the cotton. The cclumn was washed with hydrochloric acid (20 ml, 5.0 N), followed by double-distilled, de-ionized water (1.2 ml). Sodium acetate buffer (25 ml, 1.0 M), pH 6.0 was added to put the column in its usable form. A fresh column was prepared for each of the incubate samples.

The aqueous, acidic 2.0 ml sample of an incubate was introduced at the top of the Pasteur pipette and was allowed to drain through the column. The column was washed with phosphate buffer (25 ml, 0.02 M), pH 6.5, to remove L-DOPA. Elution of the dopamine was performed using hydrochloric acid (10 ml, 2.4 N). The acid eluate was titrated to pH 5 (4.9-5.2) with 6.0 N potassium carbonate, and then to approximately pH 6.3 (6.1-6.5) with 2.0 N potassium carbonate.

e. Preparation of the Dopamine Fluorophor

Conversion of dopamine to a highly fluorescing dihydroxyindole derivative was based on the method outlined by Udenfriend (1962). A volume of the neutralized extract (3.0 ml) was added to sodium acetate buffer (2.1 ml, 1.0 M). Potassium iodide (0.5 ml, 0.009 N) was then added. The colour reaction was allowed to proceed for 3 min. After that time a volume (0.5 ml) of an alkaline sulfite solution (Udenfriend, 1962) was added. After another period of 3 min, hydrochloric acid (1 ml, 5.0 N) was added. The sample stood under laboratory lighting for at least 30 min but less than twenty hours. A portion (2.0 ml) was then placed in a 3.0-ml quartz cuvette.

Fluorescence readings were performed on an Aminco-Bowman spectrophotofluorometer with the excitation and emission wavelengths set at 330 and 380 nm, respectively. These values are the uncorrected instrument settings and concur with the maximum values obtained using solutions of pure dopamine hydrochloride (13 to 520 μM) subjected to the same extraction and assay conditions as the experimental samples. In order to generate a calibration curve for the relation between dopamine concentration and fluorescence, 25 samples of known concentrations of dopamine were run through the described extraction and fluorophor generation procedures. In preliminary tests with known and experimental samples the eluates generated from each of the successive resin washes were subjected to fluorophor formation and determination. The maximum concentrations of L-DOPA, dopamine, 3-carboxysalsolinol and salsolinol theoretically possible in the incubations were examined in the extraction and fluorometric tests.

2. Formation of ^{14}C-Labelled Carbon Dioxide *In Vitro*

a. Introduction

Production of ^{14}C-labelled carbon dioxide from either (^{14}C-COOH)-3-carboxysalsolinol or (1-^{14}C)-DOPA was determined *in vitro* in the presence and in the absence of a kidney homogenate source of decarboxylase. The radioactive solutions that were used in the tests were synthesized according to the methods described earlier. Preparation of the guinea-pig kidney decarboxylase extract was performed as outlined previously.

b. Enzymatic Decarboxylation

The following reagents were pipetted into a 10-ml reaction flask (Kontes): potassium phosphate buffer (1.0 ml, 0.5M), pH 6.9; pyridoxal-5'-phosphate (0.1 ml, 1.7 mM); kidney homogenate supernatant (1.0 ml). In control tests the volume of supernatant was replaced by an equal amount of buffer. Into the centre well was pipetted NCS^{TM} (0.3 ml) tissue solubilizer (Amersham). The well stem was then inserted through a rubber cap, and the flask was capped. The vessel was placed in a shaking water bath (Grant Instruments) for 10 min at 37 C. After the equilibration either 0.10 ml of (^{14}C-COOH)-3-carboxysalsolinol (0.86 μmoles, 4.32 x 10^5 dpm) or 0.06 ml of (1-^{14}C)-DOPA (1.0 μmoles, 4.49 x 10^5 dpm) was injected through the cap into the main compartment via a 1.0-ml tuberculin syringe and a 30-gauge, 1-in needle. Control vessels contained double-distilled, de-ionized water (0.12 ml) in place of either one of the drugs. The reaction was allowed to proceed for 30 min. At the end of that time, a portion of the fluid in the centre well (0.2 ml) was removed and delivered into a glass scintillation vial containing PCS^{TM} (0.5 ml) scintillator solution (Amersham). All tests were performed in triplicate.

The level of radioactivity was determined on a Unilux II^{TM} liquid scintillation counter (Nuclear-Chicago) with ^{133}Barium as the external standard. A calibration curve for counting efficiency was determined by the channels ratio technique. Subsequently, the number of dpm occurring in the samples was calculated with the aid of a

programmed Hewlett-Packard 9821A calculator. The vials counted for a background radioactivity sampling contained NCS (0.2 ml) and of PCS (0.5 ml).

c. Nonenzymatic Decarboxylation

The technique employed was based on the method outlined by Vogel (1969). Potassium phosphate buffer (2.0 ml, 0.1 M), pH 7.5, and 0.12 ml of either (^{14}C-COOH)-3-carboxysalsolinol (0.86 μmole, 4.3 x 10^5 dpm) or double-distilled, de-ionized water were added to a 10-ml reaction flask (Kontes). Other flasks contained buffer and 0.06 ml of (1-^{14}C)-DOPA (1.0 μmole, 4.49 x 10^5 dpm). The centre well contained NCS (0.3 ml). Each flask was lowered into a water bath stabilized at 37 C. After being warmed and shaken for 2 hr, the flask was removed from the bath, and fluid from the well was rapidly removed (0.2 ml) and delivered into a glass scintillation vial containing PCS (0.5 ml). The counting of radioactivity was performed as described for the enzymatic tests. All of the tests were performed in triplicate.

3. Formation of ^{14}C-Labelled Carbon Dioxide *In Vivo*

a. Calculation of Ventilation Rate and NCS Requirement

In order to determine whether (^{14}C-COOH)-3-carboxysalsolinol could be decarboxylated *in vivo*, a technique was devised to trap the ^{14}C-labelled carbon dioxide expired by mice. Initially, calculations of respiratory rate and volume were performed in order to determine the maximal necessary quantity of the alkali trapping medium.

Guyton (1947) reported that the maximal minute volume of mice is 36 ml/min. As air contains 21% of its volume as oxygen, calculations show that the oxygen intake of a mouse amounts to 7.2 ml/min. If this intake were fully utilized, there would be an expiration of carbon dioxide at a rate of 7.2 ml/min, or 108 ml in a 15-min period at standard temperature and pressure. This latter volume at 37 C represents 4.6 mmoles of carbon dioxide.

The volume of air that is filled by carbon dioxide is less than 0.5%. The air pump employed in the experiments was fixed with a flow rate of 600 ml/min. Therefore, no more than 9 ml of carbon dioxide (0.4 mmoles) was pumped through each chamber every 15 min. Accordingly, a maximum volume of 5.0 mmoles of carbon dioxide would pass from the chamber in each 15-min period.

It was essential that this sample of carbon dioxide be trapped, by bubbling the emergent gases through alkali. NCS (250 ml, 0.6 N) can absorb 150 mmoles of carbon dioxide. Calculations show that NCS (9 ml) can absorb 5.4 mmoles of carbon dioxide, a value slightly in excess of the calculated maximum 15-min accumulations of carbon dioxide. Accordingly, this volume of NCS was placed in scintillation vials, and the emergent gases bubbled through it. Vials were changed every 15 min.

b. Collection of Expired ^{14}C-Labelled Carbon Dioxide

Male, Swiss-Webster albino mice, weighing between 25 and 28 g at the time of the experiment, were used in these experiments. The

mice were housed in colonies under a 12-hr light/12-hr dark cycle for 3 days prior to testing. They had access to water and laboratory chow *ad lib.* until 1 hr before the test was conducted.

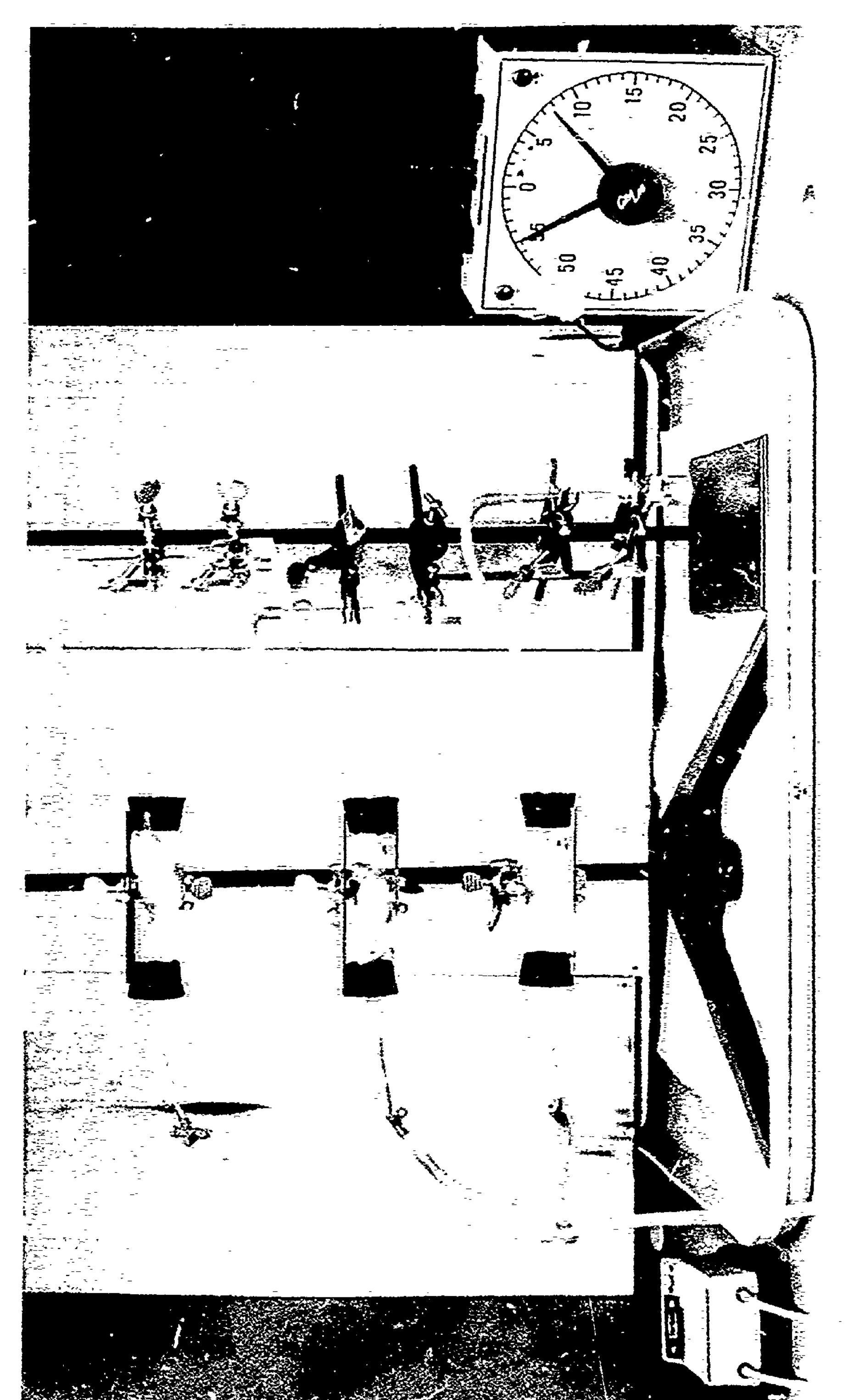
The apparatus designed for the experiments is shown in Photograph 1. A Metaframe™ Hush III air pump was calibrated to deliver air (600 ml/min) into the trapping solution. The tubing carrying the air out of the pump was connected to a plastic Y-shaped connector (Portex), which in turn was connected to latex surgical tubing. The airflow was subdivided by two plastic Y-shaped connectors (Nalgene and Portex) and latex tubing. Adjustable valves (Hykro) were inserted into the flow system to permit equal flow into each of the three test chambers. Each of the three ultimate prechamber sections of latex tubing led to a plastic straight connector (Portex), which in turn was inserted into a size 8 rubber one-hole stopper at the test chamber. The chamber was made of clear, colourless, rigid Tygon tubing, 3.7 cm ID and 13.5 cm long. At the outflow end of the chamber another stopper was inserted into the chamber end, and a plastic straight connector was attached. Latex tubing led the flow into a Pasteur pipette. The airflow emitted from the pipette was bubbled into NCS (9.0 ml) in a glass scintillation vial.

The drugs used in the tests were synthesized according to the methods described previously. Both amino acid agents, (^{14}C-COOH)-3-carboxysalsolinol and (1-^{14}C)-DOPA, were prepared in acidic saline, pH 4, and warmed to 37 C prior to intraperitoneal injection. The delivered volume was 15 μl/g body wt. The doses of the drugs were

Photograph 1

Apparatus for trapping expired ^{14}C-carbon dioxide

See Methods for details.



as follows: the more radioactive (^{14}C-COOH)-3-carboxysalsolinol, 107 μmoles Kg; the less radioactive (^{14}C-COOH)-3-carboxysalsolinol, 745 μmoles/Kg; (1-^{14}C)-DOPA, 120 μmoles/Kg. Carbidopa (102 μmoles/Kg) was delivered orally in a 5% gum acacia suspension 1 hr prior to the administration of radioactive solutions of DOPA (120 μmoles/Kg) and the isoquinoline (745 μmoles/Kg). Each mouse was used only once, and each dosage was tested three times.

All of the tests were begun at midday. Prior to being injected, the mice were acclimatized to the testing chamber for 15 min, during which time the expired air was trapped in a vial containing NCS (9.0 ml). Immediately after being injected, a mouse was placed in one of the chambers, and a second scintillation vial containing NCS (9.0 ml) replaced the preinjection vial. Every 15 min for the ensuing 2 hr, the vials were replaced with others containing NCS. Upon removal of the vial from the testing apparatus PCS (1.0 ml) was added to each vial. The vials were immediately capped and stored in a refrigerator (4 C) until assayed in the scintillation counter. Each series of scintillation readings was performed as described previously on the same afternoon as the test in which its samples were generated.

Behavioural changes in mice receiving the radiolabelled compounds were observed. Qualitative results were recorded.

4. Statistical Analysis of Decarboxylation Tests

For the results of the dopamine formation tests, a calibration curve for known amounts of the amine was determined using the linear

regression analysis programme 100-2-ST3 of the Wang 600 Series calculator. The concentration of dopamine formed in an experimental sample could then be estimated from the curve.

The data generated in the ^{14}C-carbon dioxide formation tests were subjected to analysis on the calculator. For any given test, programme number 1014-2-ST1 was used to estimate the mean, variance and standard error of the mean. Student's two-tailed t-test for the significant difference of the means was computed through the use of programme number 1010-2-ST2. The results were considered to be significant at $P < 0.05$.

V RESULTS

A. Behavioural Experiments

1. Ethanol-induced Narcosis

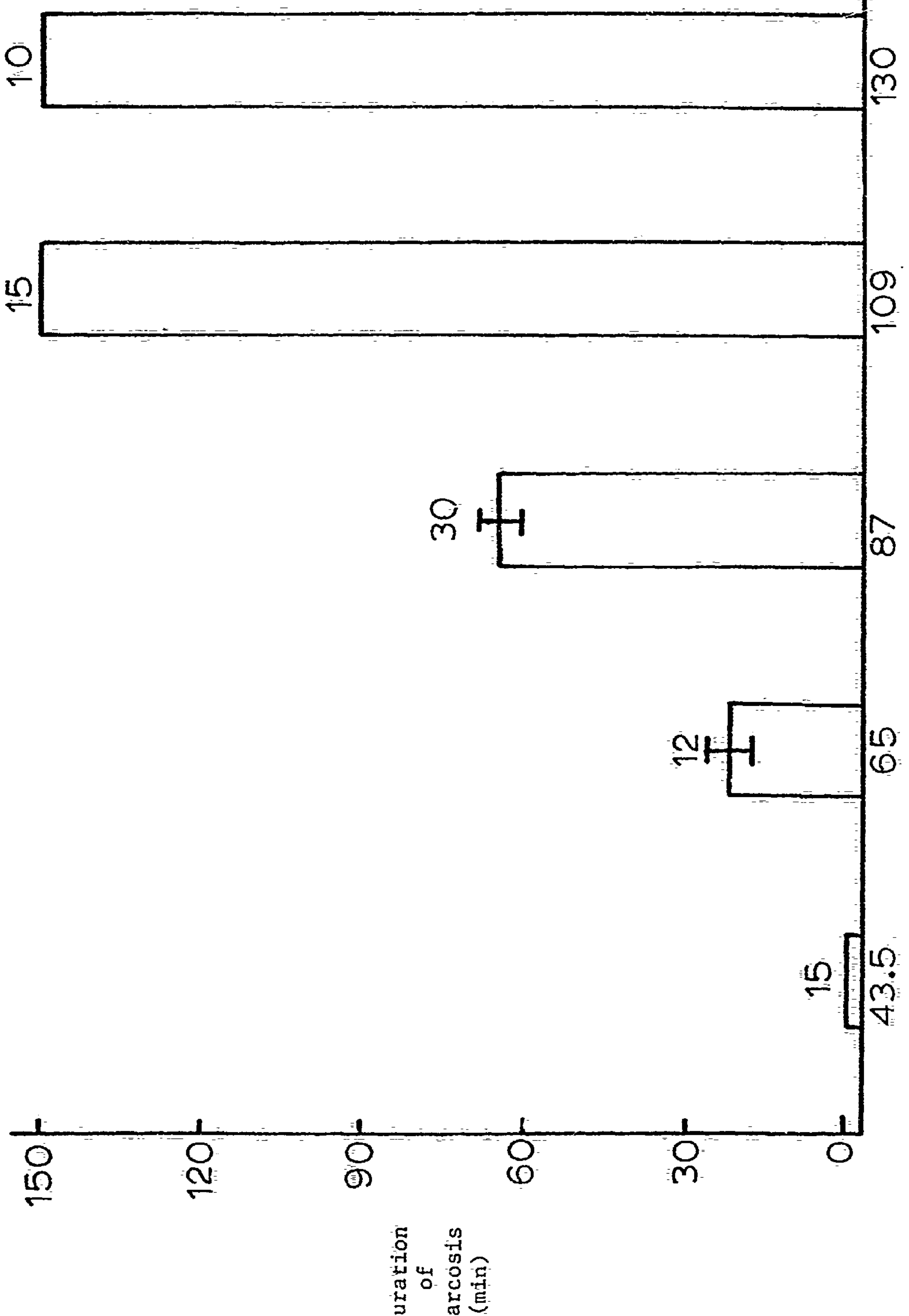
Mice injected with ethanol (87 mmoles/Kg) lost the ability to right themselves from a supine position. The duration of this narcosis was altered by prior or concomitant treatment with certain other drugs.

Results of the tests in which ethanol (43.5 to 174 mmoles/Kg) was the sole agent are illustrated in Fig. 3. The lowest dose did not produce a loss of the righting reflex in any one of 15 mice. With the higher concentrations of ethanol the severity of the loss increased in a dose-dependent manner. The duration recorded for ethanol in a concentration of 87 mmoles/Kg was obtained by combining the results of three tests, which did not differ significantly from one another. One group of mice had been injected with saline immediately before ethanol was given. Another group had received acidic saline, and the third group had no concomitant treatment. All of the mice that had been administered ethanol (109 mmoles/Kg) remained supine for 150 min. A similar result was observed for ethanol (130 mmoles/Kg), with an 80% mortality rate by 24 hr later. Within 30 min after the administration of the highest concentration (174 mmoles/Kg) all ten mice died.

Figure 3

Duration of ethanol-induced narcosis

A solution of ethanol, 25% (v/v), was injected intraperitoneally in the doses shown. All 10 mice injected with ethanol in a dose of 174 mmoles/Kg (8 g/Kg) died within 30 min after treatment. The number of subjects in each test is illustrated above the respective error bars.


Duration of narcosis (min)
0
30
60
90
120
150
Dose of ethanol (mmoles/Kg)
43.5
65
87
109
130
15
12
30
15
10

Treatment with either L-DOPA (1000 μ moles/Kg) or dopamine (60 μ moles/Kg) lengthened the duration of ethanol-induced narcosis significantly. Lower doses of L-DOPA produced no significant change. The results of the L-DOPA-ethanol and dopamine-ethanol experiments are shown in Fig. 4. When either L-DOPA or dopamine was injected intraperitoneally or intravenously in a concentration of 60 μ moles/Kg in the absence of ethanol, no loss of righting reflex occurred, and neither ataxia nor any related behavioural alteration was apparent.

Intraperitoneal administration of either 3-carboxysalsolinol or salsolinol (60 μ moles/Kg) produced no observable change in behaviour. Treatment with dimethoxysalsolinol (60 μ moles/Kg) led to a slight hyperactivity, but this was not quantified. Neither 3-carboxysalsolinol nor salsolinol in a concentration of 60 μ moles/Kg yielded observable effects when the drugs were delivered intravensouly.

Fig. 5 shows the changes produced by isoquinoline treatments given in conjunction with ethanol. The dose-response patterns generated by 3-carboxysalsolinol and salsolinol were biphasic, the lowest and highest doses producing shorter durations than the intervening doses.

The administration of 3-carboxysalsolinol (15, 30 or 60 μ moles /Kg) prolonged ethanol-induced narcosis significantly. Maximum prolongation of the narcosis was produced by 3-carboxysalsolinol in a dose of 60 μ moles/Kg. Salsolinol led to a significant increase in narcosis duration, its greatest effect occurring in a

Figure 4

Alteration of the duration of ethanol-induced narcosis
by L-DOPA and dopamine

The drugs were injected intraperitoneally immediately prior to injections of ethanol (87 mmoles/Kg). The results were compared with the value obtained with saline treatment and ethanol.

$n = 10$ unless illustrated otherwise

a $P < 0.05$

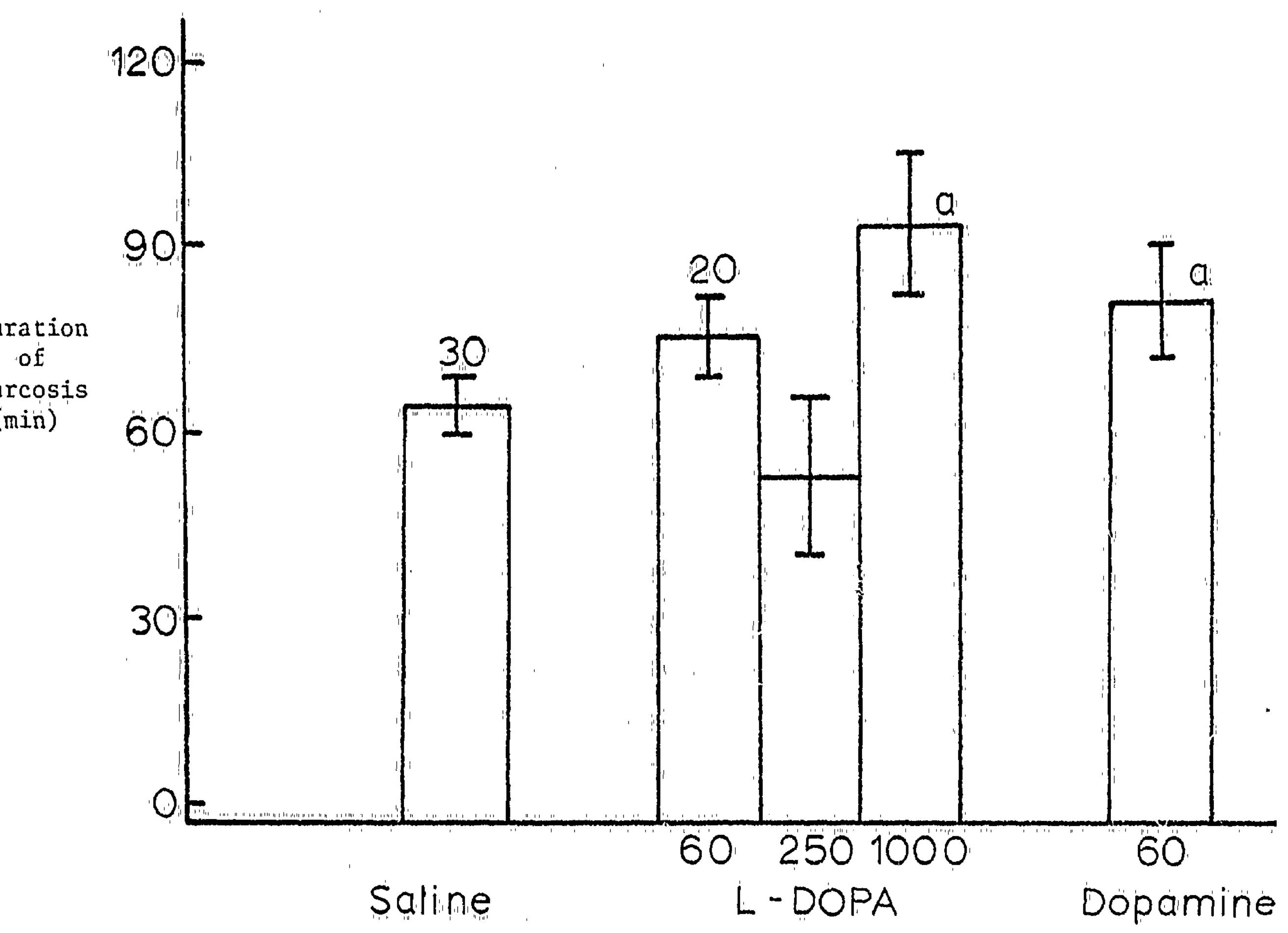

Duration of narcosis (min)
0
30
60
90
120
30
20
a
a
60
250
1000
60
Saline
L - DOPA
Dopamine
Drug treatment (μmoles/Kg) with ethanol (87 mmoles/Kg)

Figure 5

Alteration of the duration of ethanol-induced narcosis by 3-carboxysalsolinol, salsolinol and dimethoxysalsolinol

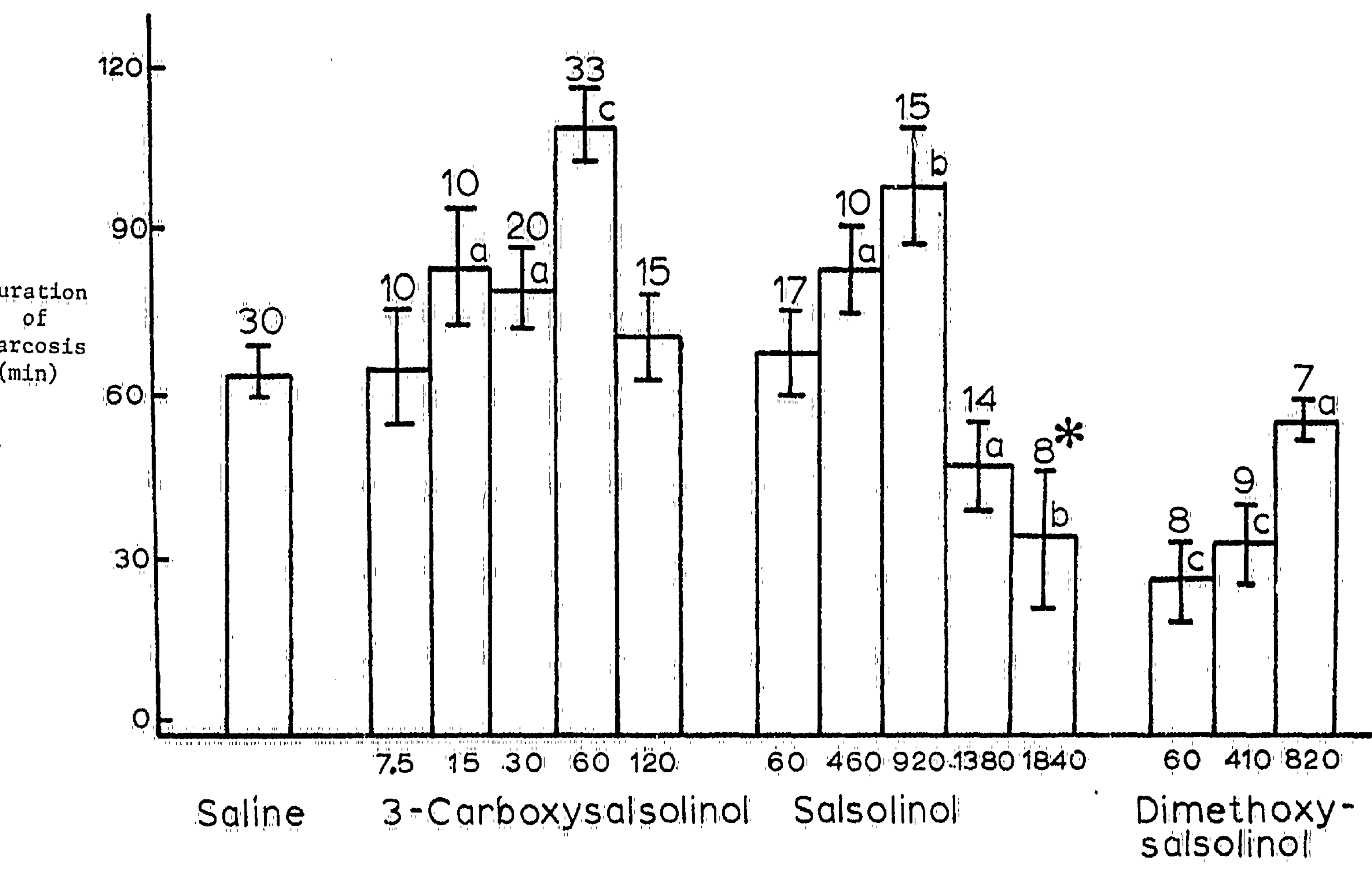
The drugs were injected intraperitoneally immediately prior to injections of ethanol (87 mmoles/Kg). The results were compared with the value obtained with saline and ethanol.

* 8 of the 20 mice administered salsolinol (1840 μmols/Kg) with ethanol lost the righting reflex for various lengths of time. The other 12 mice died within 30 min after the injection of ethanol.

a $P < 0.05$

b $P < 0.01$

c $P < 0.001$


Duration of narcosis (min)
120
90
60
30
0
30
10
10
a
20
a
33
c
15
17
10
a
15
b
14
a
8*
b
8
c
9
c
7
a
7.5
15
30
60
120
60
460
920
1380
1840
60
410
820
Saline
3-Carboxysalsolinol
Salsolinol
Dimethoxy-salsolinol
Drug treatment (μmoles/kg) with ethanol (87 mmoles/kg)

concentration of 920 μ moles/Kg. When salsolinol (1380 μ moles/Kg) was administered with ethanol, the narcosis duration was greatly shortened as compared with the saline-ethanol control value. In a concentration of 1840 μ moles/Kg salsolinol given with ethanol was lethal. More than half of the mice so injected died within 30 min. The surviving subjects remained supine for 11 to 78 min. Treatment with dimethoxysalsolinol (60 or 410 μ moles/Kg) attenuated the duration of the ethanol-induced reflex loss. Increasing the dose of dimethoxysalsolinol to 820 μ moles/Kg led to significantly less attenuation of the response. Even so, this effect was still significant in comparison with the control value.

Fig. 6 illustrates the results obtained with the ADH inhibitor pyrazole administered in combination with other agents. When pyrazole (0.5 mmole/Kg) was given 30 min before treatment with saline and ethanol the duration of sleep was significantly longer than with saline pretreatment. A higher dose of pyrazole slightly enhanced the response. Neither L-DOPA nor dopamine significantly altered the effect produced by ethanol with pyrazole pretreatment, but treatments with the isoquinolines did lead to noticeable changes. When 3-carboxysalsolinol was given, the pyrazole-ethanol response was lengthened significantly. Salsolinol (60 μ moles/Kg) did not change the pyrazole-ethanol value, but a higher dose greatly decreased it.

Fig. 7 shows the results of narcosis tests in which disulfiram, an inhibitor of AldDH and DBH, was delivered 24 hr before other drugs. The narcosis induced by ethanol in the presence of

Figure 6

Alteration of the duration of ethanol-induced narcosis by pyrazole and amines

Pyrazole (0.5 or 1.0 mmoles/Kg) was administered intraperitoneally 1 hr before treatment with ethanol (87 mmoles/Kg). The results were compared with the value obtained with saline pretreatment. The amines were injected immediately prior to injections of ethanol and 1 hr after pyrazole (0.5 mmoles/Kg). These results were compared with the values obtained with pyrazole (0.5 mmoles/Kg) and ethanol.

XXX Saline pretreatment

PYR Pyrazole

DA Dopamine

3cSAL 3-Carboxysalsolinol

SAL Salsolinol

n = 10 unless illustrated otherwise

a $P < 0.05$ b $P < 0.01$

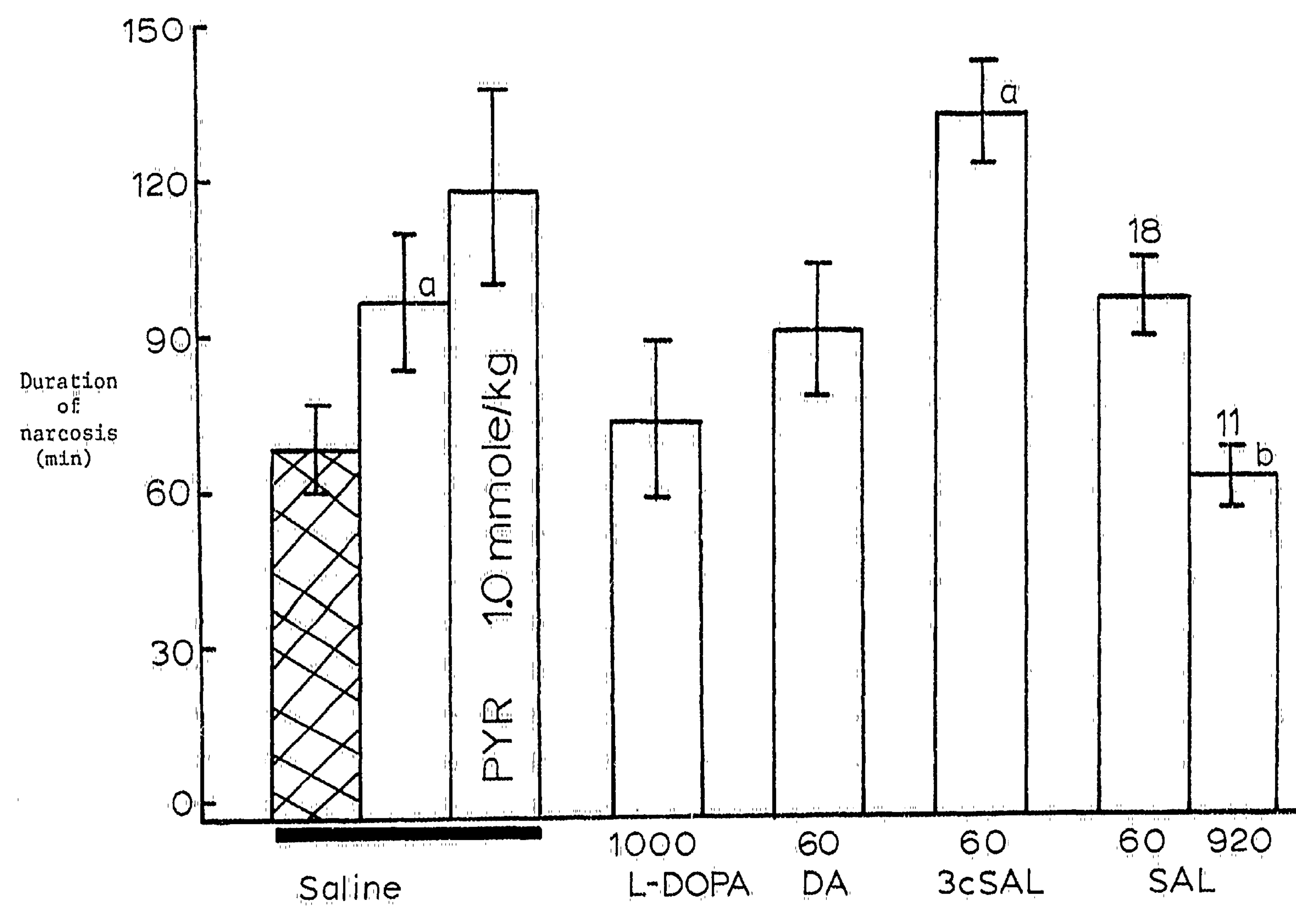

150
120
90
60
30
0
Duration of narcosis (min)
a
PYR 1.0 mmole/kg
a
18
11
b
Saline
1000
L-DOPA
60
DA
60
3cSAL
60 920
SAL
Drug treatment (μmoles/Kg) with ethanol (87 mmoles/Kg)

Figure 7

Alteration of the duration of ethanol-induced narcosis
by disulfiram and amines

Disulfiram (0.25, 0.5 and 1.01 mmoles/Kg) was administered orally as a suspension in 5% gum acacia 24 hr before treatment with ethanol (87 mmoles/Kg). The results were compared with the value obtained with acacia pretreatment. The amines were injected immediately prior to injections of ethanol and 24 hr after disulfiram (0.25 mmoles/Kg). These results were compared with the value obtained with disulfiram (0.25 mmoles/Kg) and ethanol.

XXX Acacia pretreatment

DS Disulfiram

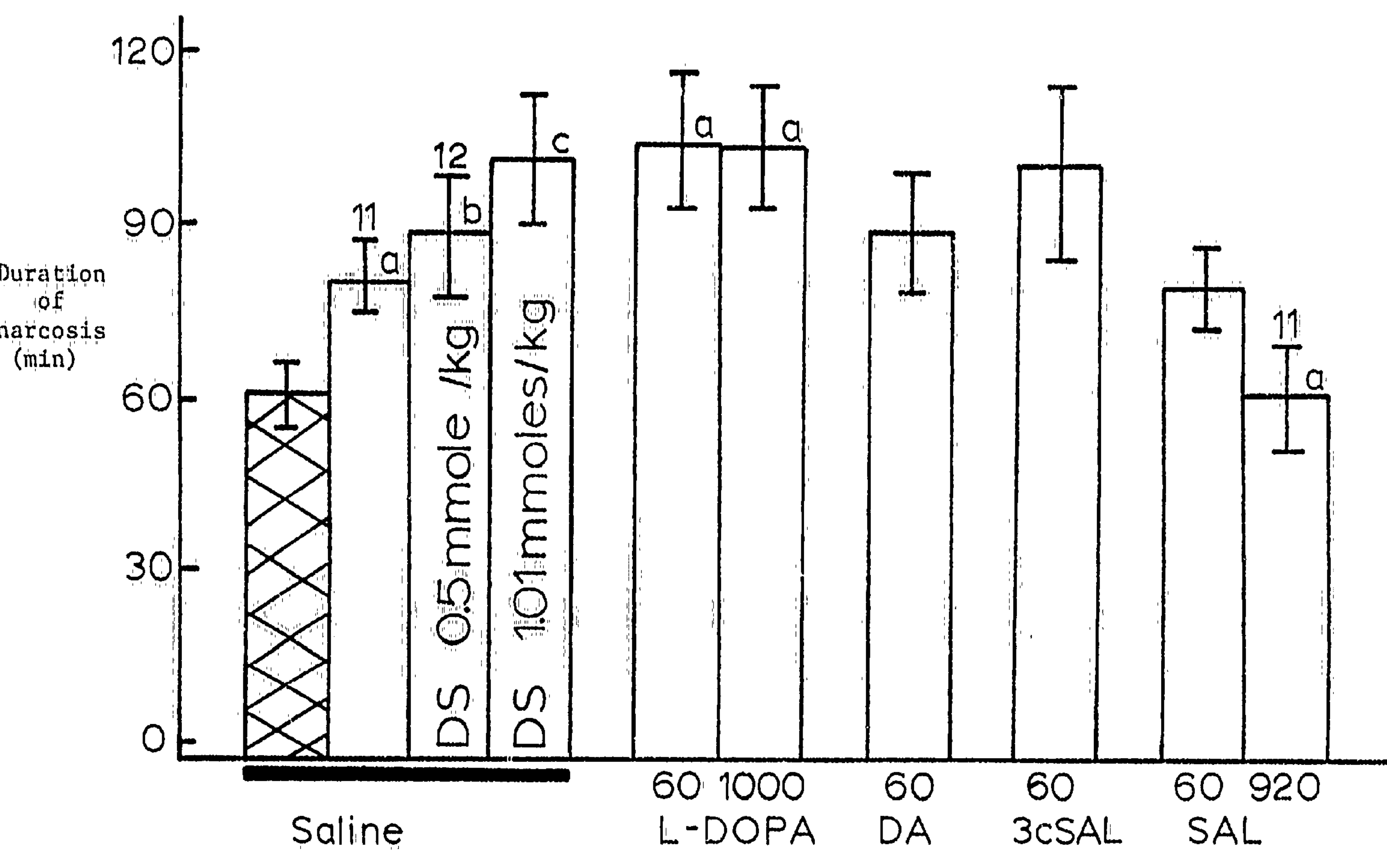
DA Dopamine

3cSAL 3-Carboxysalsolinol

SAL Salsolinol

$n = 10$ unless illustrated otherwise

a $P < 0.05$ b $P < 0.01$ c $P < 0.001$



Drug treatment (μmoles/Kg) with ethanol (87 mmoles/Kg)

cotreatment with saline was increased by disulfiram in a dose-dependent manner. Significantly longer durations of narcosis were achieved with the higher disulfiram doses. Treatment with L-DOPA enhanced the disulfiram (0.25 mmole/Kg)-ethanol response significantly. Dopamine, 3-carboxysalsolinol or salsolinol at 60 μ moles/Kg had no effect on the disulfiram-ethanol result. When a higher dose of salsolinol was administered, the disulfiram-ethanol narcosis value was shortened.

Some narcosis tests were conducted with the MAO inhibitor pargyline given 4 hr before other agents. The results of these tests are illustrated in Fig. 8. Pargyline (51 or 102 μmoles/Kg) increased slightly the duration of the response observed with ethanol and saline. A higher dose of pargyline profoundly lengthened the duration of ethanol-induced narcosis. The pargyline-ethanol response was not altered significantly by L-DOPA, dopamine or the isoquinolines.

Fig. 9 illustrates the observations made in narcosis tests involving inhibition of peripheral L-amino-acid decarboxylase by carbidopa. When carbidopa was delivered 1 hr before treatment with ethanol and saline no change in the narcosis duration was observed. The lowest dose of L-DOPA did not alter the carbidopa-ethanol response. Higher doses of L-DOPA significantly prolonged the response. 3-Carboxysalsolinol given with ethanol in the presence of carbidopa pretreatment resulted in a narcosis duration significantly longer than that observed for carbidopa with ethanol.

Figure 8

Alteration of the duration of ethanol-induced narcosis by pargyline and amines

Pargyline (51, 102 or 510 μmoles/Kg) was administered intraperitoneally 4 hr before treatment with ethanol (87 mmoles/Kg). The results were compared with the values obtained with saline pretreatment. The ami es were injected immediately prior to injections of ethanol and 4 hr after pargyline (51 μmoles/Kg). These results were compared with the value obtained with pargyline (51 μmoles/Kg) and ethanol.

XXX Saline pretreatment

PARG Pargyline

DA Dopamine

3cSAL 3-Carboxysalsolinol

SAL Salsolinol

$n = 10$ unless illustrated otherwise

c $P < 0.001$

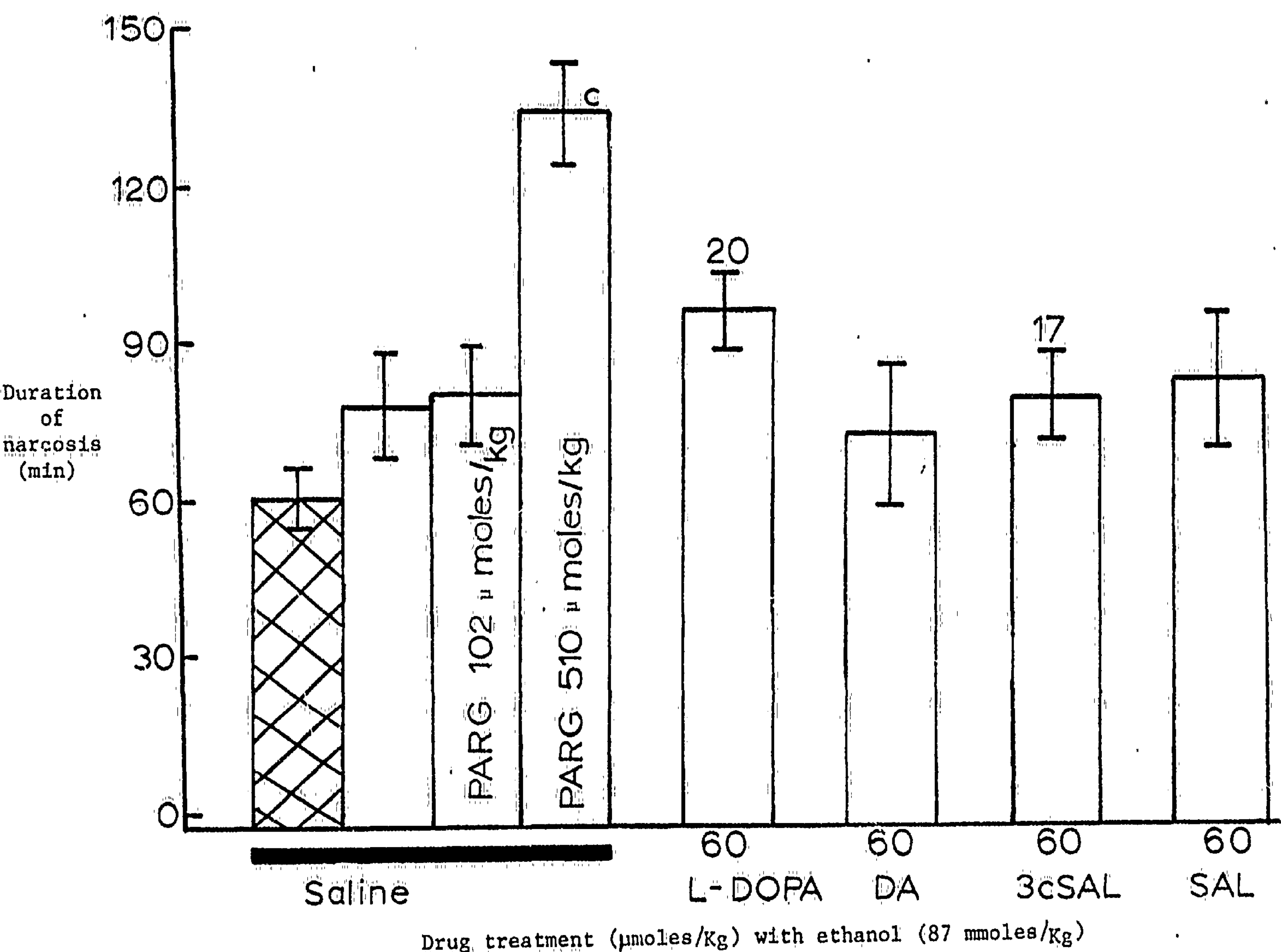

150
120
90
60
30
0
Duration of narcosis (min)
c
20
17
PARG 102 µ moles/kg
PARG 510 µ moles/kg
Saline
60
L-DOPA
60
DA
60
3cSAL
60
SAL
Drug treatment (µmoles/Kg) with ethanol (87 mmoles/Kg)

Figure 9

Alteration of the duration of ethanol-induced narcosis by carbidopa and amino acids

Carbidopa (102 umoles/Kg) was administered orally as a suspension in 5% gum acacia 1 hr before treatment with ethanol (87 mmoles/Kg). The result was compared with the value obtained with acacia pretreatment. The amino acids were injected immediately prior to injections of ethanol and 1 hr after carbidopa. These results were compared with the value obtained with carbidopa and ethanol.

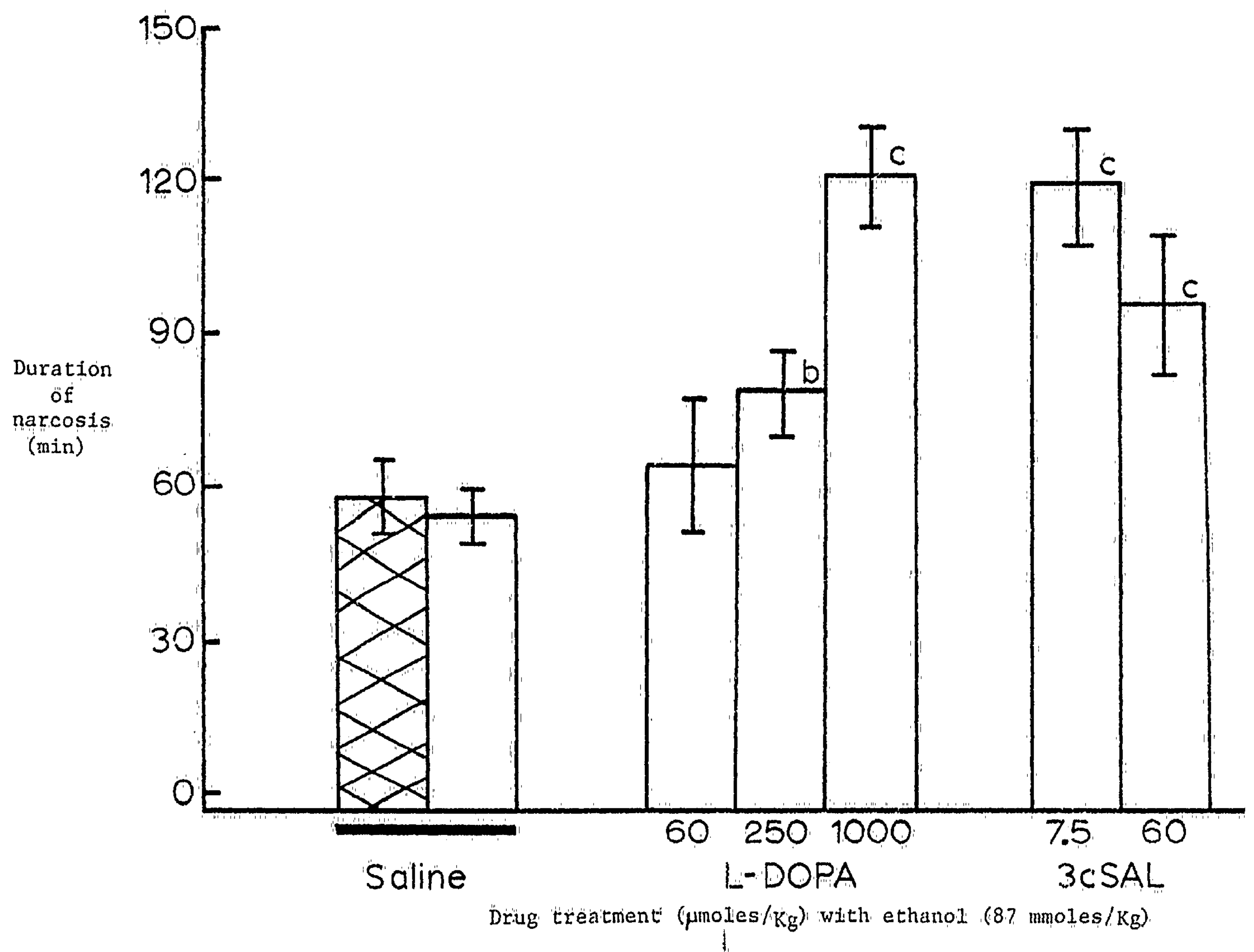
XXX Acacia pretreatment

3cSAL 3-Carboxysalsolinol

$n = 10$

b $P < 0.01$

c $P < 0.001$



150
120
90
60
30
0
Duration of narcosis (min)
c
c
c
b
60
250
1000
7.5
60
Saline
L-DOPA
3cSAL
Drug treatment (μmoles/Kg) with ethanol (87 mmoles/Kg)

In some experiments benserazide was employed as an inhibitor of centrally and peripherally located L-amino-acid decarboxylase. The results of these studies are shown in Fig. 10. Injections of saline and ethanol 1 hr after benserazide administration produced a narcosis response equalling that occurring after saline pretreatment. L-DOPA (250 μmoles/Kg) did not change the response arising from treatment with benserazide and ethanol. Upon administration of a higher dose of L-DOPA the narcosis duration evident for benserazide and ethanol was greatly prolonged. The two lowest doses of the isoquinoline 3-carboxysalsolinol did not alter the duration of narcosis generated by benseraxide with ethanol. At a higher dosage of 3-carboxysalsolinol the duration was shortened.

The lack of an effect of the dopamine receptor blocker haloperidol is illustrated in Fig. 11. This agent produced no significant change in the duration of the loss of the righting reflex at any of the concentrations employed prior to ethanol administration.

2. Hexobarbital-induced Narcosis

Treatment with hexobarbital induced a loss of the righting reflex in mice. The duration of the barbiturate narcosis was not altered by agents given in the dose ranges that did affect ethanol narcosis.

Fig. 12 shows that neither L-DOPA nor 3-carboxysalsolinol significantly changed the duration of the hexobarbital-induced sleep, as compared with saline cotreatment. The decarboxylase inhibitor carbidopa given before saline and hexobarbital yielded a

Figure 10

Alteration of the duration of ethanol-induced narcosis by benserazide and amino acids

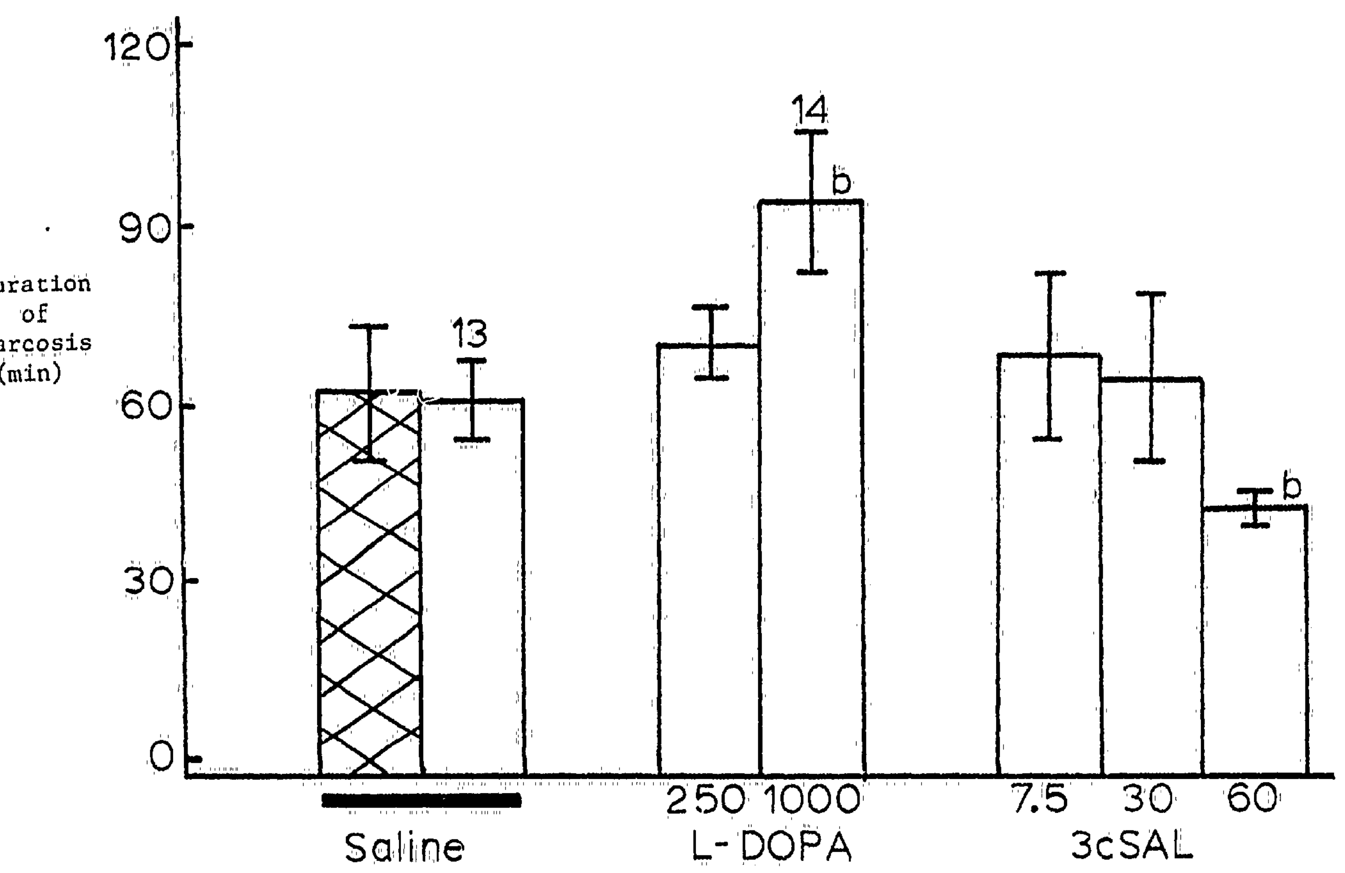
Benserazide (1.7 mmoles/Kg) was administered intraperitoneally 1 hr before treatment with ethanol (87 mmoles/Kg). The result was compared with the value obtained with saline pretreatment. The amino acids were injected immediately prior to injections of ethanol and 1 hr after benserazide. These results were compared with the value obtained with benserazide and ethanol.

XXX Saline pretreatment

3cSAL 3-Carboxysalsolinol

$n = 10$ unless illustrated otherwise

b $P < 0.01$



Drug treatment (μmoles/Kg) with ethanol (87 mmoles/Kg)

Figure 11

Alteration of the duration of ethanol-induced narcosis by haloperidol

Haloperidol (75, 150 or 300 nmoles/Kg) was administered intraperitoneally 3 hr before treatment with ethanol (87 mmoles/Kg). The results were compared with the value obtained with saline pretreatment.

n = 10

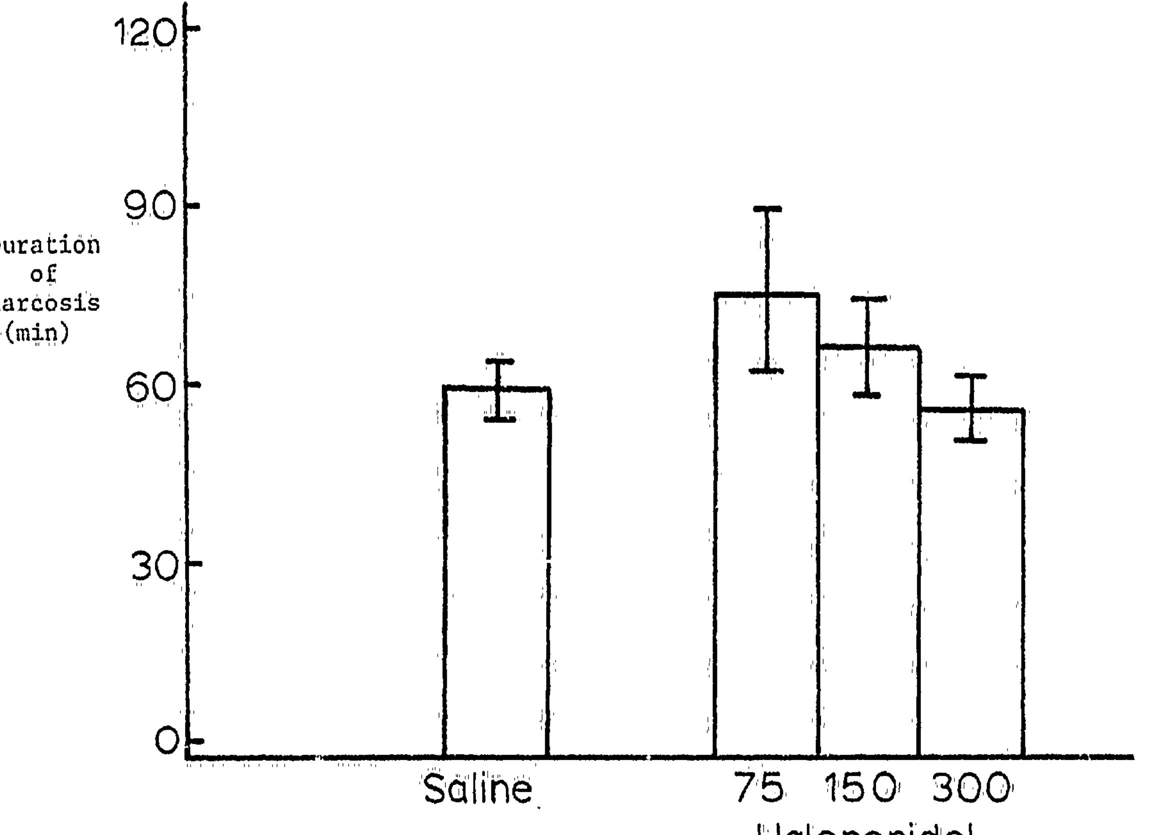

120
90
60
30
0
Duration
of
narcosis
(min)
Saline
75 150 300
Haloperidol
Drug pretreatment (nmoles/Kg) with ethanol (87 mmoles/Kg)

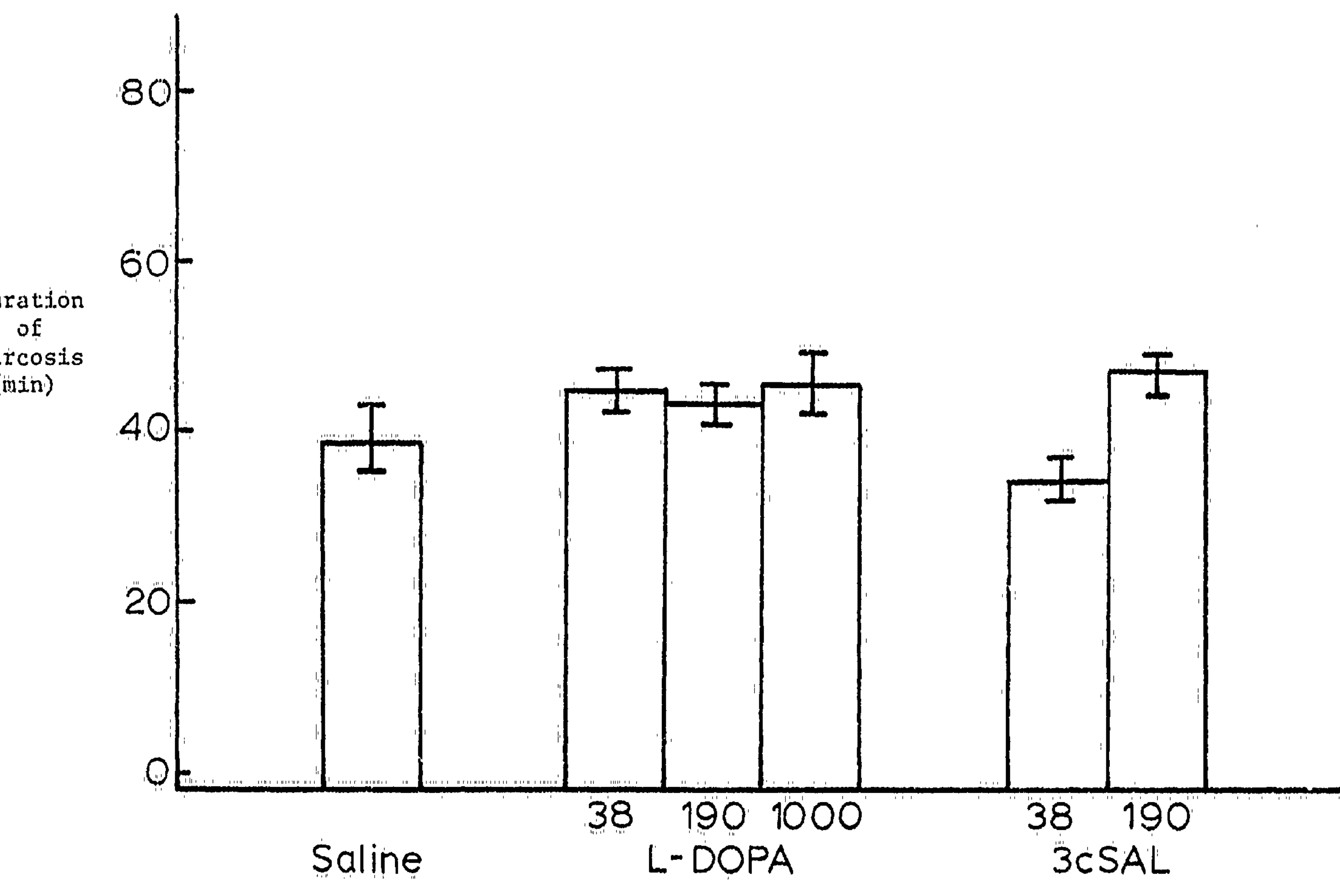
Figure 12

Alteration of the duration of hexobarbital-induced narcosis by amino acids

The drugs were injected intraperitoneally immediately prior to injections of hexobarbital (0.39 mmoles/Kg). The results were compared with the value obtained with saline and ethanol.

3cSAL 3-Carboxysalsolinol

$n = 10$


Duration
of
narcosis
(min)
80
60
40
20
0
38
190
1000
38
190
Saline
L-DOPA
3cSAL
Drug treatment (µmoles/Kg) with hexobarbital (0.39 mmoles/Kg)

response comparable to that resulting from acacia pretreatment. These observations are illustrated in Fig. 13, as are the lack of affects of L-DOPA and 3-carboxysalsolinol on the responses.

3. Tail-clip Analgesia

The percentage of mice demonstrating analgesia in response to the placing of a tail-clip was determined at different times after treatment with 3-carboxysalsolinol given in different doses. Similar analgesia tests were conducted with the isoquinoline in the presence of pretreatment with the decarboxylase inhibitor carbidopa. In analogous experiments L-DOPA was administered in the absence or presence of carbidopa pretreatment. Some analgesia tests involved the administration of the amino acid compounds in combination with morphine, and decarboxylase inhibitors were given in certain of these tests. Naloxone blockade of analgesia produced by the various agents and combinations of agents was also examined.

The results of tests in which analgesia was scored at various times after treatment with 3-carboxysalsolinol are shown in Table 1. The data indicate that the greatest percentage of mice that were analgesic occurred 30 min after 3-carboxysalsolinol (220 μmoles/Kg) was injected. Although not shown, a subsequent test revealed minimal analgesia 15 min after injection of 3-carboxysalsolinol (220 μmoles/Kg): only 5% of the mice were analgesic at that time. With carbidopa pretreatment incorporated into the protocol, it was demonstrated that 3-carboxysalsolinol (4.4 μmoles/Kg) was most effective (40%) at 30 min. In Fig. 14 the responses occurring at 30 min for different

Figure 13

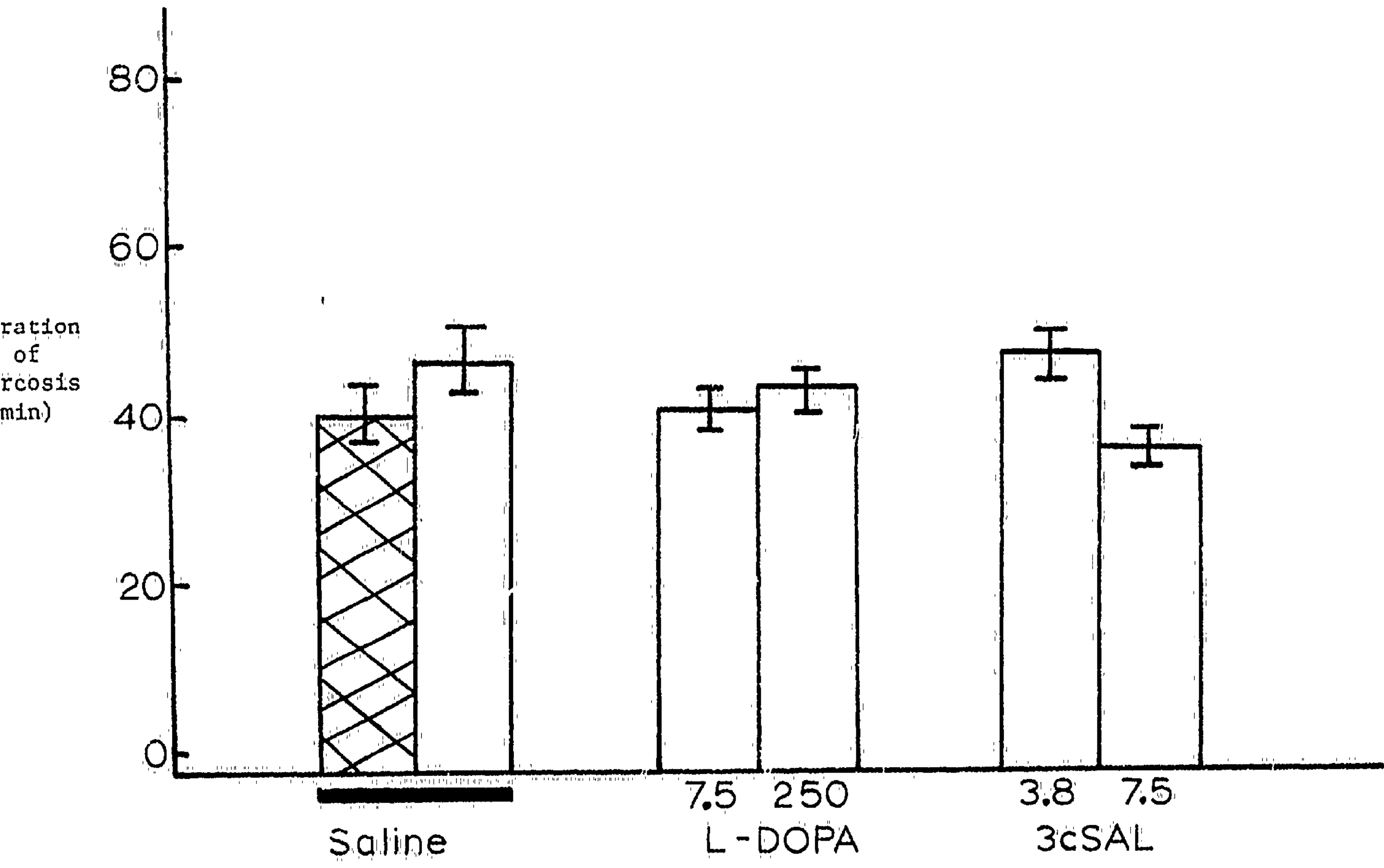
Alteration of the duration of hexobarbital-induced narcosis by carbidopa and amino acids

Carbidopa (102 μmoles/Kg) was administered orally as a suspension in 5% gum acacia 1 hr before treatment with hexobarbital (0.39 mmoles/Kg). The result was compared with the value obtained with acacia pretreatment. The amino acids were injected immediately prior to injections of hexobarbital and 1 hr after carbidopa. The results were compared with the value obtained with carbidopa and ethanol.

XXX Acacia pretreatment

3cSAL 3-Carboxysalsolinol

n = 10



Drug treatment with hexobarbital (0.39 mmoles/Kg)

Table 1

Analgesic effects of 3-carboxysalsolinol *

Dose of 3-carboxysalsolinol (μmoles/Kg)	Percentage of mice displaying analgesia at 30	60	90	120	150 min
0	3	5	5	0	-
44	15	10	10	10	-
110	21	25	20	10	10
220	54	35	20	10	15
440	30	0	10	15	0
880	24	10	10	5	-
1.1**	5	0	0	0	-
4.4**	40	30	25	15	10

*with 10-sec limit in the tail-clip test

**mice pretreated with carbidopa (102 μmoles/Kg) 1 hr before 3-carboxysalsolinol

n = 30 at 30 min; n = 20 at remaining times

Figure 14

Log dose-response curves of the analgesic effect of 3-carboxysalsolinol with and without carbidopa

The responses of mice to the placing of a tail-clip were recorded 30 min after the intraperitoneal drug injections. A latency to response of 10 sec was chosen as the limit. The results were compared with the value obtained with saline.

$n = 30$

X Saline

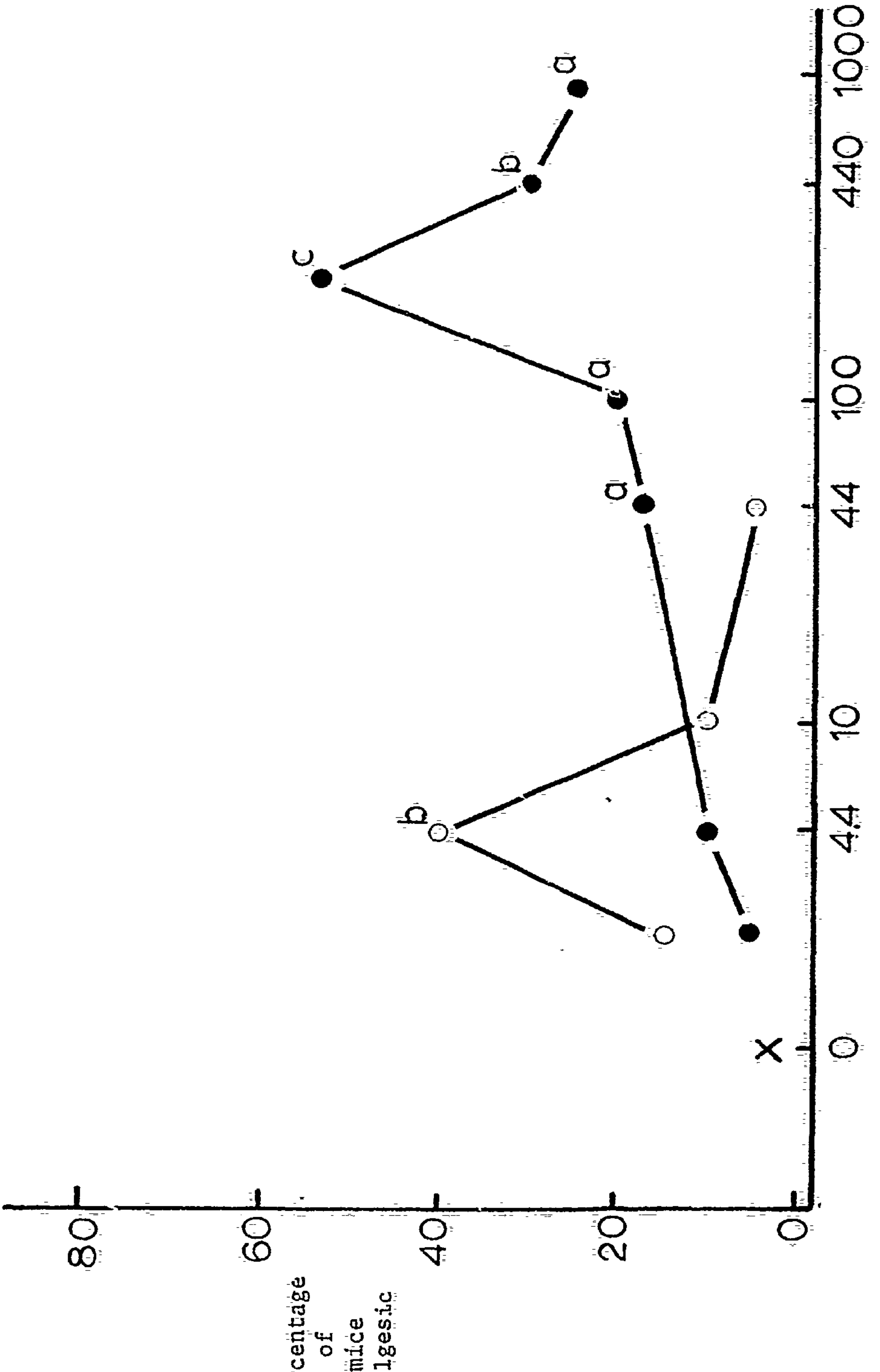
●— 3-carboxysalsolinol alone

○— 3-carboxysalsolinol administered 1 hr after carbidopa (102 μmoles/Kg) given orally as a suspension in 5% gum acacia

a $P < 0.05$

b $P < 0.02$

c $P < 0.01$


Percentage
of
mice
analgesic
0
20
40
60
80
X
0
4.4
10
44
100
440
1000
b
a
a
c
b
a
Dose of 3-carboxysalsolinol (µmoles/Kg)

doses of 3-carboxysalsolinol in the absence or presence of carbidopa are shown. The biphasic dose-response patterns are evident. It was observed also that carbidopa on its own produced no analgesic effect. In the absence of carbidopa the responses arising from each of several doses of 3-carboxysalsolinol were significant with respect to the saline control. In the presence of carbidopa, only the result occurring with 3-carboxysalsolinol in a concentration of 4.4 μmoles/Kg was significant in comparison with the responses obtained without carbidopa.

Fig. 15 illustrates that L-DOPA administration produced low analgesia percentages, two of which were significant as compared with saline values. Carbidopa pretreatment did not increase significantly the value obtained with L-DOPA (4.4 μmoles/Kg).

The effect of treatment with 3-carboxysalsolinol on morphine-induced analgesia is shown in Fig. 16. Dose increases of morphine, resulted in increases in the number of mice displaying analgesia. When 3-carboxysalsolinol (44 μmoles/Kg) was administered in place of saline, the resulting response to each of the three different doses of morphine was not significantly altered. In a higher concentration, 3-carboxysalsolinol increased significantly the analgesic response to morphine (11 μmoles/Kg). But the isoquinoline did not change the response to either of the higher morphine doses. Pretreatment with carbidopa had no significant effect on the percentage of mice showing analgesia induced by the administration of the lowest dose of morphine. Combining 3-carboxysalsolinol (4.4 μmoles/Kg) into

Figure 15

Log dose-response curve of the analgesic effect of L-DOPA with and without carbidopa

The responses of mice to the placing of a tail-clip were recorded 30 min after the intraperitoneal drug injections. A latency to response of 10 sec was chosen as the limit. The results were compared with the value obtained with saline.

$n = 30$

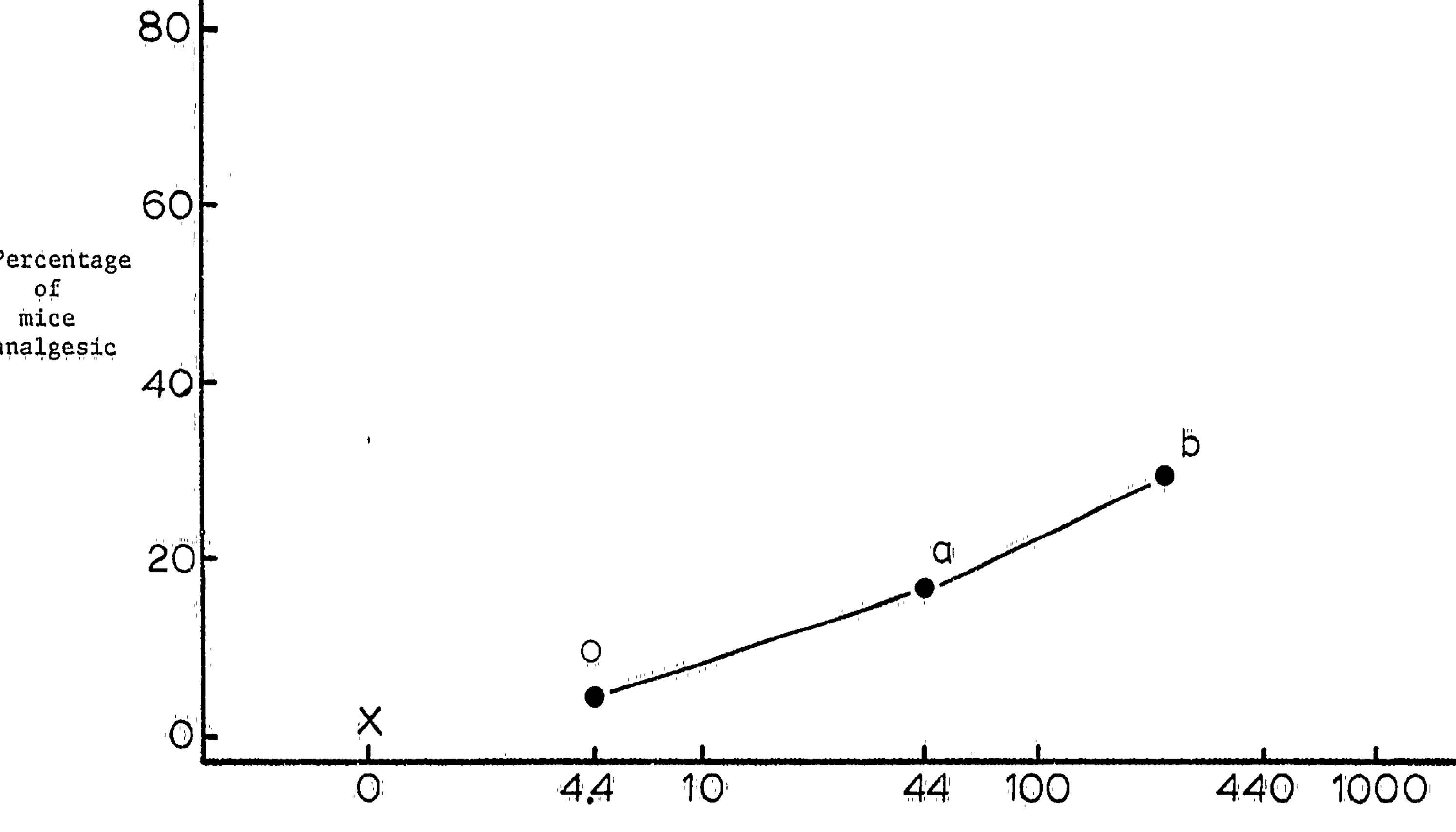
X Saline

●— L-DOPA

○ L-DOPA administered 1 hr after carbidopa (102 μmoles/Kg) given orally as a suspension in 5% gum acacia

a $P < 0.05$

b $P < 0.02$



80
60
40
20
0
Percentage of mice analgesic
X
o
a
b
0
4.4
10
44
100
440
1000
Dose of L-DOPA (μmoles/Kg)

Figure 16

Alteration of morphine-induced analgesia by 3-carboxysalsolinol

The responses of mice to the placing of a tail-clip were recorded 30 min after the intraperitoneal drug injections. A latency to response of 10 sec was chosen as the limit. 3-Carboxysalsolinol was administered immediately prior to the injections of morphine. The results were compared with the values obtained with the respective doses of morphine without 3-carboxysalsolinol.

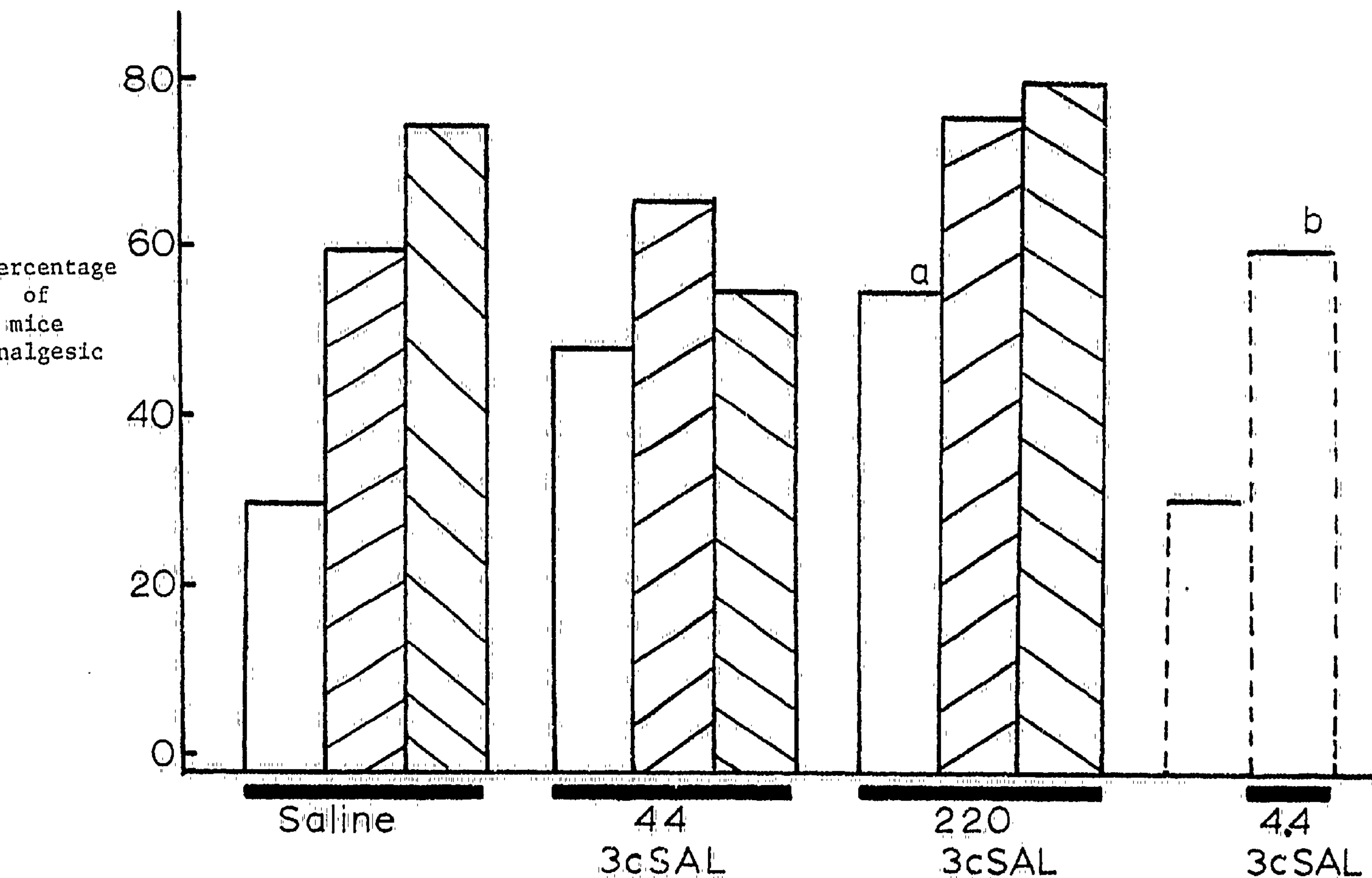
3cSAL 3-Carboxysalsolinol

Morphine (μmoles/Kg): [open bar], 11; [hatched bar], 16.5; [cross-hatched bar], 22; [dashed bar], 11, 1 hr after carbidopa (102 μmoles/Kg) given orally as a suspension in 5% gum acacia

$n = 30$

a $P < 0.05$

b $P < 0.02$


Percentage
of
mice
analgesic
80
60
40
20
0
a
b
Saline
44
3cSAL
220
3cSAL
4.4
3cSAL
Dose of 3-carboxysalsolinol (μmoles/Kg) with morphine

this protocol led to a significantly increased number of mice showing analgesia.

In concentrations equimolar with those of 3-carboxysalsolinol, L-DOPA did not alter significantly any of the analgesia responses observed with saline and morphine. Analgesia scores after the administration of L-DOPA and morphine are given in Fig. 17. The result arising from the lowest dose of morphine in the presence of carbidopa was not changed by treatment with L-DOPA.

Fig. 18 shows that naloxone abolished the analgesia resulting from treatment with saline, carbidopa and 3-carboxysalsolinol, 3-carboxysalsolinol alone, and L-DOPA. Responses generated by morphine in the presence of the amino acid compounds were also abolished by naloxone. These responses, illustrated in Fig. 19, arose from the following combinations of agents: saline and morphine; 3-carboxysalsolinol and morphine; L-DOPA and morphine.

4. Spontaneous Locomotor Activity

The spontaneous locomotor activity of mice at the beginning of a 12 hr cycle of darkness was assessed. Upon injection of 3-carboxysalsolinol on the following evening, movements were again counted, and were compared with the scores of the baseline activity of the previous evening.

The baseline activities recorded for mice which received drug injections 24 hr later are shown in Appendix 2. The ratios of the number of movements recorded after injection of 3-carboxysalsolinol

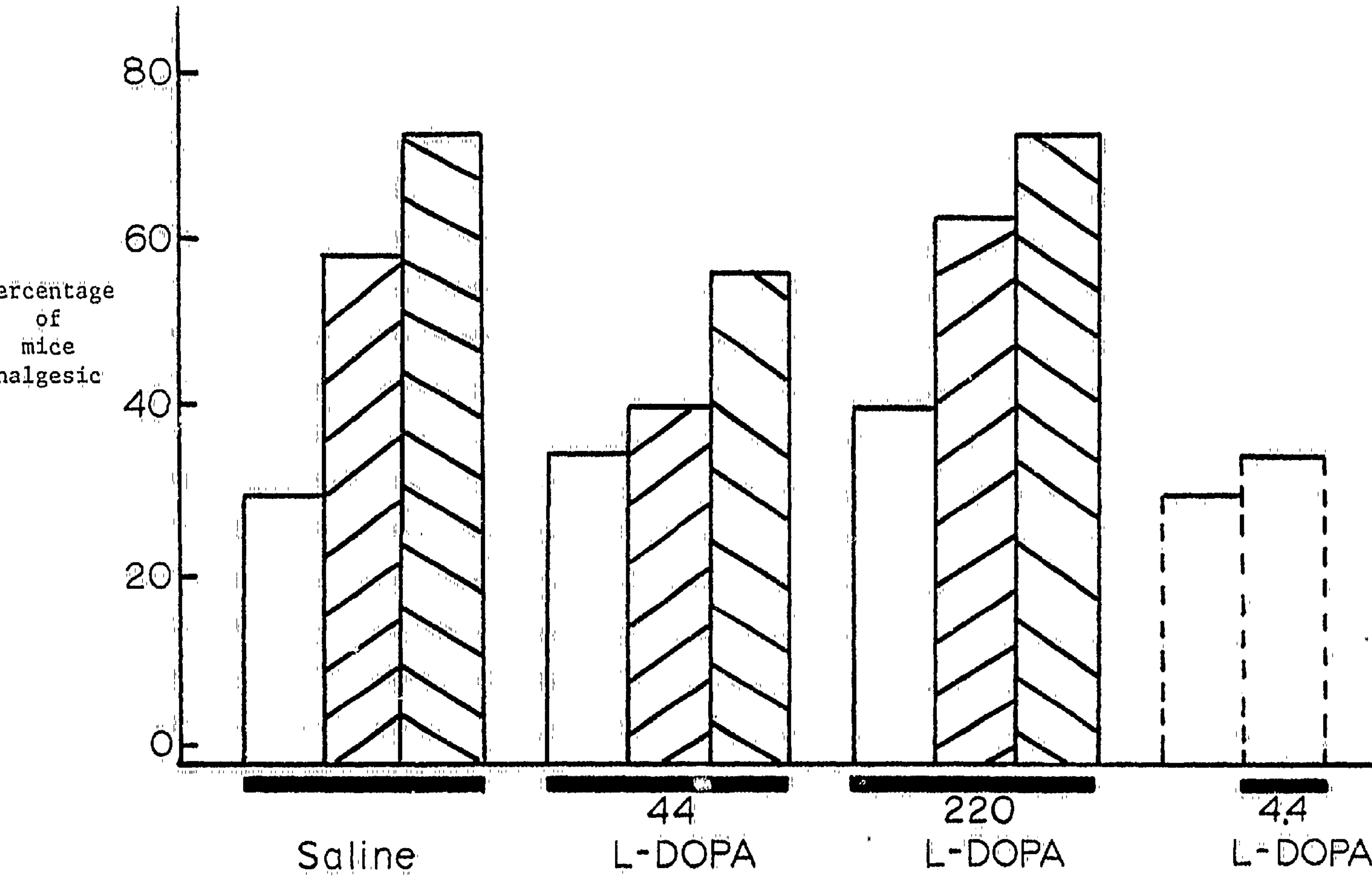

80
60
40
20
0
Percentage
of
mice
analgesic
Saline
44
L-DOPA
220
L-DOPA
4.4
L-DOPA
Dose of L-DOPA (μmoles/Kg) with morphine

Figure 18

Alteration of 3-carboxysalsolinol- and L-DOPA-induced analgesia by naloxone

The responses of mice to the placing of a tail-clip were recorded 30 min after the intraperitoneal drug injections. A latency to response of 10 sec was chosen as the limit. Naloxone was administered immediately prior to the injections of the analgesic.

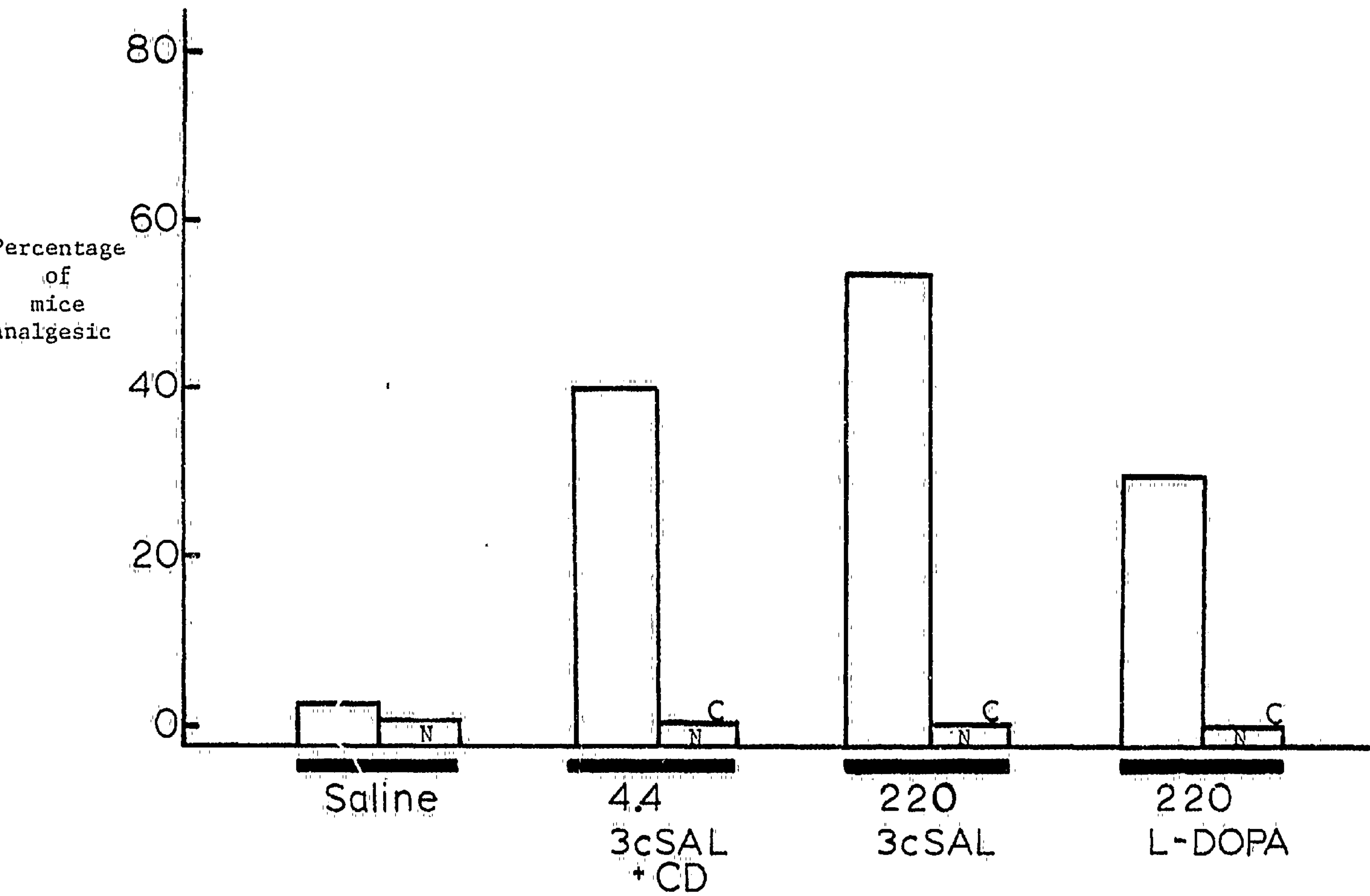
$n = 30$

n = Naloxone

3cSAL 3-Carboxysalsolinol

CD Carbidopa (102 μmoles/Kg) given orally in 5% gum acacia 1 hr prior to 3-carboxysalsolinol

$p < 0.001$



Percentage of mice analgesic
80
60
40
20
0
N
C
N
C
N
C
N
Saline
4.4
3cSAL
+CD
220
3cSAL
220
L-DOPA
Dose of analgesic drug (μmoles/Kg)

Figure 19

Alteration by naloxone of analgesia
produced by morphine with amines

The responses of mice to the placing of a tail-clip were recorded 30 min after the intraperitoneal drug injections. A latency of response of 10 sec. was chosen as the limit. Naloxone was administered immediately prior to the injections of the analgesics.

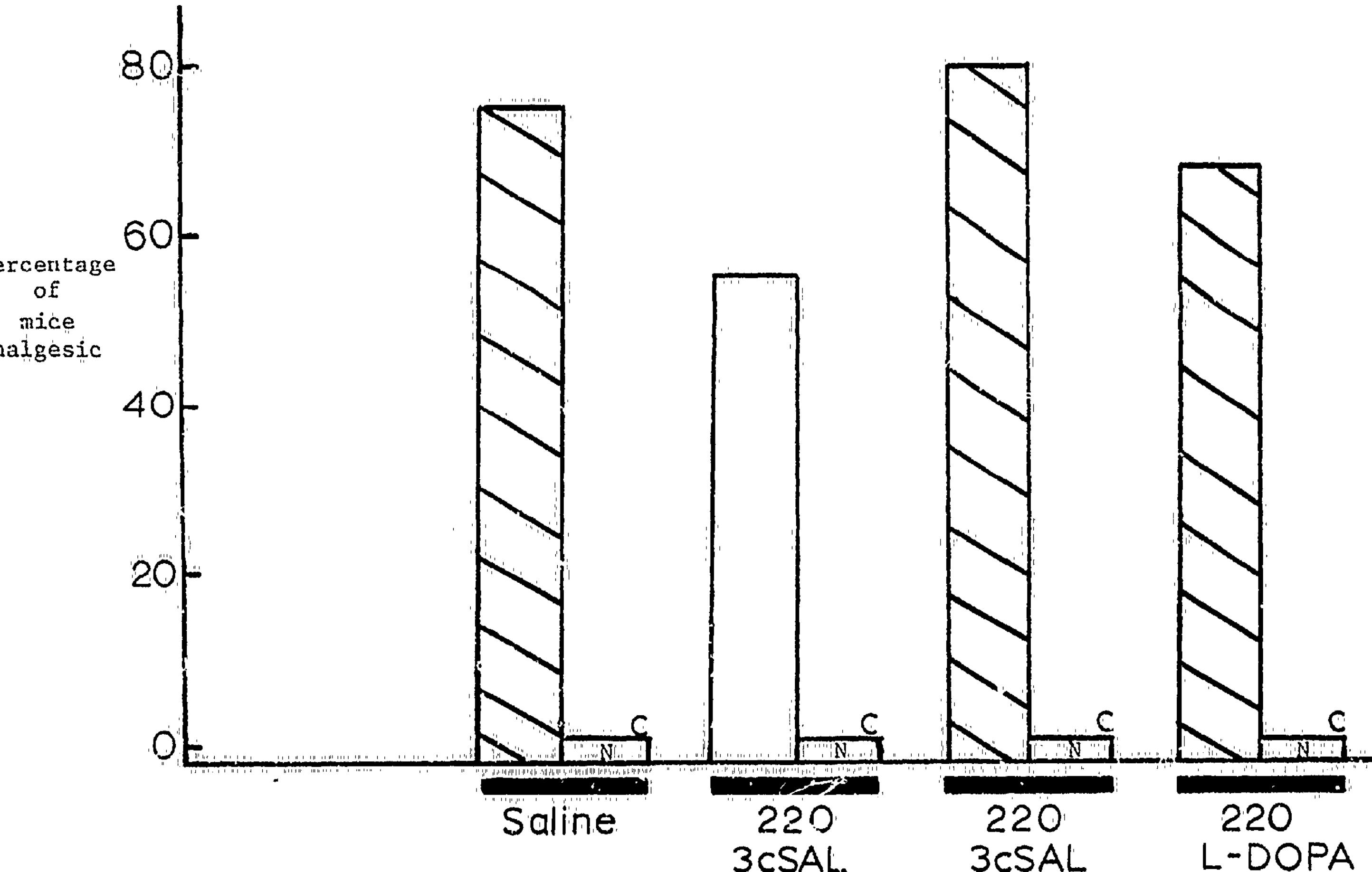
n = 30

N Naloxone

3cSAL 3-Carboxysalsolinol

Morphine (μ moles/Kg): [open box], 11; [hatched box], 22;

c $P < 0.001$



Drug treatment (μmoles/Kg) with morphine

or saline to the number of movements recorded as baseline activity are shown in Table 2. Various comparisons of the tests were made in order to determine the noteworthy results.

As seen in the top row of values in Table 2, the lowest dose of 3-carboxysalsolinol produced significantly more activity than did saline at 12 min, at 36 min and especially at 60 min after injection. A higher dose of 3-carboxysalsolinol did not yield results significantly different from saline for any of the five recorded intervals. At 60 min after the administration of the highest dose of 3-carboxysalsolinol the spontaneous locomotor activity was significantly less than that recorded for saline. Comparison of the two lower doses of 3-carboxysalsolinol demonstrated that the 60 min values were significantly different, the lowest dose producing the most activity. Comparison of the highest and lowest concentrations of 3-carboxysalsolinol indicated that at 12, 36 and 60 min the highest dose produced less activity than the lowest. No significant difference in activity was apparent for any of the five comparisons made between the highest and intermediate doses.

B. Decarboxylation Experiments

1. Alteration of Dopamine and Carbon Dioxide Formation *In Vitro*

The enzymatic formation of dopamine from L-DOPA in the presence and in the absence of 3-carboxysalsolinol was determined. Known concentrations of dopamine hydrochloride were carried through

Table 2

Comparisons of spontaneous locomotor activity after injection with baseline activity.

3-Carboxysalsolinol (μmoles/Kg) Test A	vs	Test B	Ratios (± S.E.M.) of activities at 6 - 12 A	B	30 - 36 A	B	54 - 60 A	B	90 - 96 A	B	114 - 120 min A	B
38		0	1.01 ±0.136	0.67 ±0.071	1.15 ±0.190	0.49 ±0.104	1.24 ±0.106	0.51 ±0.119	0.82 ±0.131	1.01 ±0.167	1.01 ±0.048	0.92 ±0.128
			$P < 0.05$		$P < 0.02$		$P < 0.01$		N.S.		N.S.	
100		0	0.59 ±0.188	0.67 ±0.071	0.69 ±0.243	0.49 ±0.104	0.29 ±0.150	0.51 ±0.119	0.78 ±0.139	1.01 ±0.167	0.64 ±0.213	0.92 ±0.128
			N.S.		N.S.		N.S.		N.S.		N.S.	
190		0	0.64 ±0.083	0.67 ±0.071	0.28 ±0.087	0.49 ±0.104	0.10 ±0.034	0.51 ±0.119	0.71 ±0.185	1.01 ±0.167	1.01 ±0.165	0.92 ±0.128
			N.S.		N.S.		$P < 0.02$		N.S.		N.S.	
100		38	0.59 ±0.188	1.01 ±0.136	0.69 ±0.243	1.15 ±0.190	0.29 ±0.150	1.24 ±0.106	0.78 ±0.139	0.82 ±0.131	0.64 ±0.213	1.01 ±0.048
			N.S.		N.S.		$P < 0.01$		N.S.		N.S.	
190		38	0.64 ±0.083	1.01 ±0.136	1.28 ±0.087	1.15 ±0.190	0.10 ±0.034	1.24 ±0.106	0.71 ±0.185	0.82 ±0.131	1.01 ±0.165	1.01 ±0.048
			$P < 0.05$		$P < 0.01$		$P < 0.001$		N.S.		N.S.	
190		100	0.64 ±0.083	0.59 ±0.188	0.28 ±0.087	0.69 ±0.243	0.10 ±0.034	0.29 ±0.150	0.71 ±0.185	0.78 ±0.139	1.01 ±0.165	0.64 ±0.213
			N.S.		N.S.		N.S.		N.S.		N.S.	

N.S., not significant at $P \leq 0.05$

the extraction procedures and fluorescence assay. A calibration curve relating the intensity of fluorescence to the concentration of dopamine was generated. Upon extraction and assay of experimental samples, the resulting fluorescence readings were used to determine, from the calibration curve, the concentration of dopamine in each sample. Initially, the percentage recovery of dopamine from the resin was determined. Regression analysis of the line showing the relation between fluorescence readings and known concentrations of dopamine not entered onto the resin columns indicated a slope of 0.75 ± 0.001 and a correlation coefficient of 0.992. Regression analysis of 25 samples of dopamine entered onto resin is represented in Fig. 20. The slope of the line is 1.01 ± 0.001 with a correlation coefficient of 0.975. Fluorescence values were greater after resin extraction than in the absence of resin. The pair of values for 390 μM dopamine differed significantly ($P < 0.01$). A high percentage recovery of dopamine was inferred but not determined.

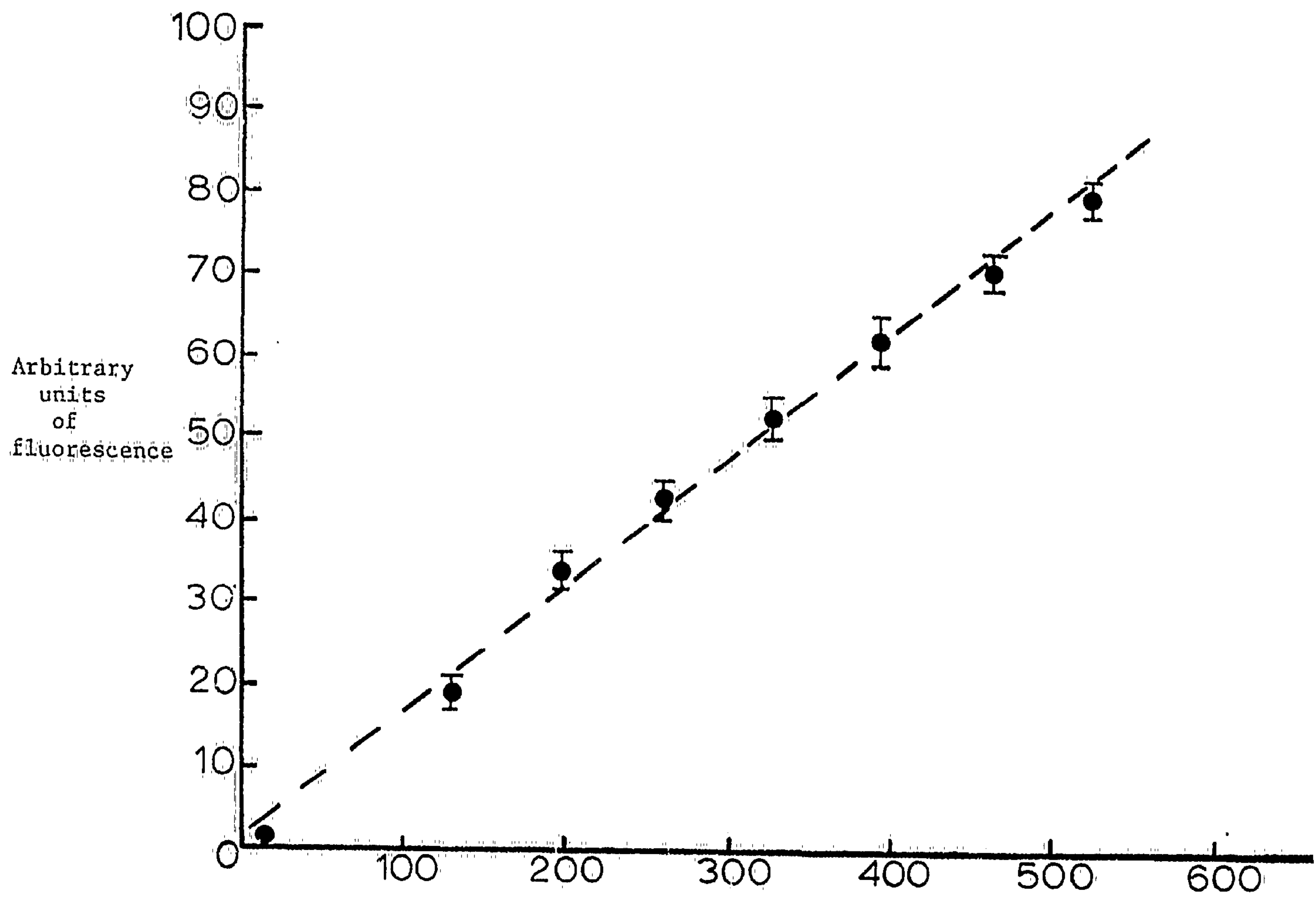
Table 3 shows the fluorescence readings resulting from the conversion of L-DOPA in the presence and in the absence of 3-carboxysalsolinol. Without enzyme or without substrate the response was negligible. Samples from the flasks containing L-DOPA produced a significant amount of fluorescence. As read from the resin-generated calibration curve, this amount of fluorescence corresponds to a concentration of dopamine of approximately 275 μM, or 0.64 μmole of dopamine per flask. Vessels containing 3-carboxysalsolinol but without L-DOPA yielded no significant amount of fluorescence. Some of the flasks contained both L-DOPA and the carboxylated isoquinoline. It was observed that this combination of agents produced significantly

Figure 20

Fluorescence calibration curve of resin-treated dopamine

$r = 0.975$

$slope = 1.01 \pm 0.001$



100
90
80
70
60
50
40
30
20
10
0
Arbitrary units of fluorescence
100
200
300
400
500
600
Concentration of dopamine (μM)

Table 3

Alteration of L-DOPA decarboxylation by 3-carboxysalsolinol *in vitro*

L-DOPA (μmoles/flask)	3cS (μmoles/flask)	Dopamine (μmoles/flask)	CO_2 (μmoles/flask)
0	0	0.02 ± 0.007	0.01 ± 0.008
0	0.50	0.02 ± 0.001	0.01 ± 0.006
*1.0	0	0.03 ± 0.002	0.02 ± 0.006
1.0	0	0.64 ± 0.021	0.38 ± 0.042
1.0	0.25	0.81 ± 0.043	0.60 ± 0.007
1.0	0.50	0.94 ± 0.019	0.76 ± 0.065

(n = 9)

*no enzyme

greater amounts of fluorescence than that generated by L-DOPA alone. The response observed for the L-DOPA-3-carboxysalsolinol (0.25 μmole) combination corresponds to approximately 350 μM dopamine, or 0.81 μmole of dopamine per flask. The response for L-DOPA with carboxysalsolinol (0.5 μmole) was slightly over 400 μM dopamine, or 0.94 μmole of dopamine per flask.

Volume changes were measured manometrically, and the volumes of liberated carbon dioxide were converted into micromoles. The values are presented in Table 3. In flasks containing L-DOPA in the presence of enzyme homogenate, approximately 0.38 μmole carbon dioxide was generated. The addition of 3-carboxysalsolinol and L-DOPA to the flask led to significantly greater production of carbon dioxide. Negligible fluorescence responses observed for the enzyme blank and the substrate blanks paralleled the negligible pressure responses recorded from flasks containing these controls.

2. Formation of ^{14}C-Labelled Carbon Dioxide *In Vitro*

By extending the decarboxylase incubation technique to (^{14}C-COOH)-3-carboxysalsolinol and (1-^{14}C)-DOPA, it was possible to determine the production of ^{14}C-labelled carbon dioxide from the amino compounds. Variations of pH and duration of incubation were incorporated into the protocol.

Table 4 illustrates the results of the radioactive studies performed *in vitro*. When (^{14}C-COOH)-3-carboxysalsolinol was incubated in the presence of enzyme at pH 6.9 for 30 min, 0.82%

Table 4

Decarboxylation of (^{14}C-COOH)-3-carboxysalsolinol and (1-^{14}C)-DOPA *in vitro*

A. pH 6.9, 30 minutes incubation, n = 3:

Substrate (μmoles/flask)	Enzyme Presence	No. of dpm Collected	Conversion (%)
Water	Yes	61 ± 8.5	---
(^{14}C-COOH)-3cS (0.86)	Yes	3,548 ± 406	0.82 ± 0.094
(^{14}C-COOH)-3cS (0.86)	No	1,364 ± 295	0.32 ± 0.068
(1 - ^{14}C)-DOPA (1.0)	Yes	133,877 ± 3,562	29.90 ± 0.820
(1 - ^{14}C)-DOPA (1.0)	No	4,395 ± 157	1.01 ± 0.036

B. pH 7.5, 120 minutes incubation, n = 3:

Substrate (μmoles/flask)	Enzyme Presence	No. of dpm Collected	Conversion (%)
Water	No	99 ± 7.1	---
(^{14}C-COOH)-3cS (0.86)	No	4,911 ± 1,931	1.14 ± 0.044
(1 - ^{14}C)-DOPA (1.0)	No	28,143 ± 549	6.25 ± 0.122

3cS 3-Carboxysalsolinol

counting efficiency = 91%

of the radioactivity of the compound was converted to ^{14}C-labelled carbon dioxide. In the absence of enzyme this conversion was 0.32%. These two conversion percentages differed significantly ($P < 0.05$). Both values were greatly above the background level obtained for the water control. The conversion of (^{14}C-COOH)-DOPA in the presence of decarboxylase was 29.9%. In the absence of enzyme a 1.01% conversion was obtained. These values are significantly different. At a higher pH and with a longer incubation nonenzymatic formation of ^{14}C-labelled carbon dioxide was apparent. For (^{14}C-COOH)-3-carboxy-salsolinol the conversion was 1.14% and for (1-^{14}C)-DOPA, 6.25%. These values differ significantly from each other.

3. Formation of ^{14}C-Labelled Carbon Dioxide *In Vivo*

a. Scintillation Measurements

As described in the Methods section of this thesis, after injection of (^{14}C-COOH)-3-carboxysalsolinol or (1-^{14}C)-DOPA mice were housed in chambers that permitted the collection of ^{14}C-labelled carbon dioxide expired by the mice. Alkali was used to trap the carbon dioxide, and the radioactivity present in these samples was assayed.

Fig. 21 shows the ^{14}C-carbon dioxide collected after injection of the lower dose of (^{14}C-COOH)-3-carboxysalsolinol. The first sample taken contained the largest amount of ^{14}C-carbon dioxide, and there was a progressive decline in radioactivity in the remaining 15 min samples. All of the radioactivity readings were significantly greater than the background count of 137 ± 11.0 dpm.

Figure 21

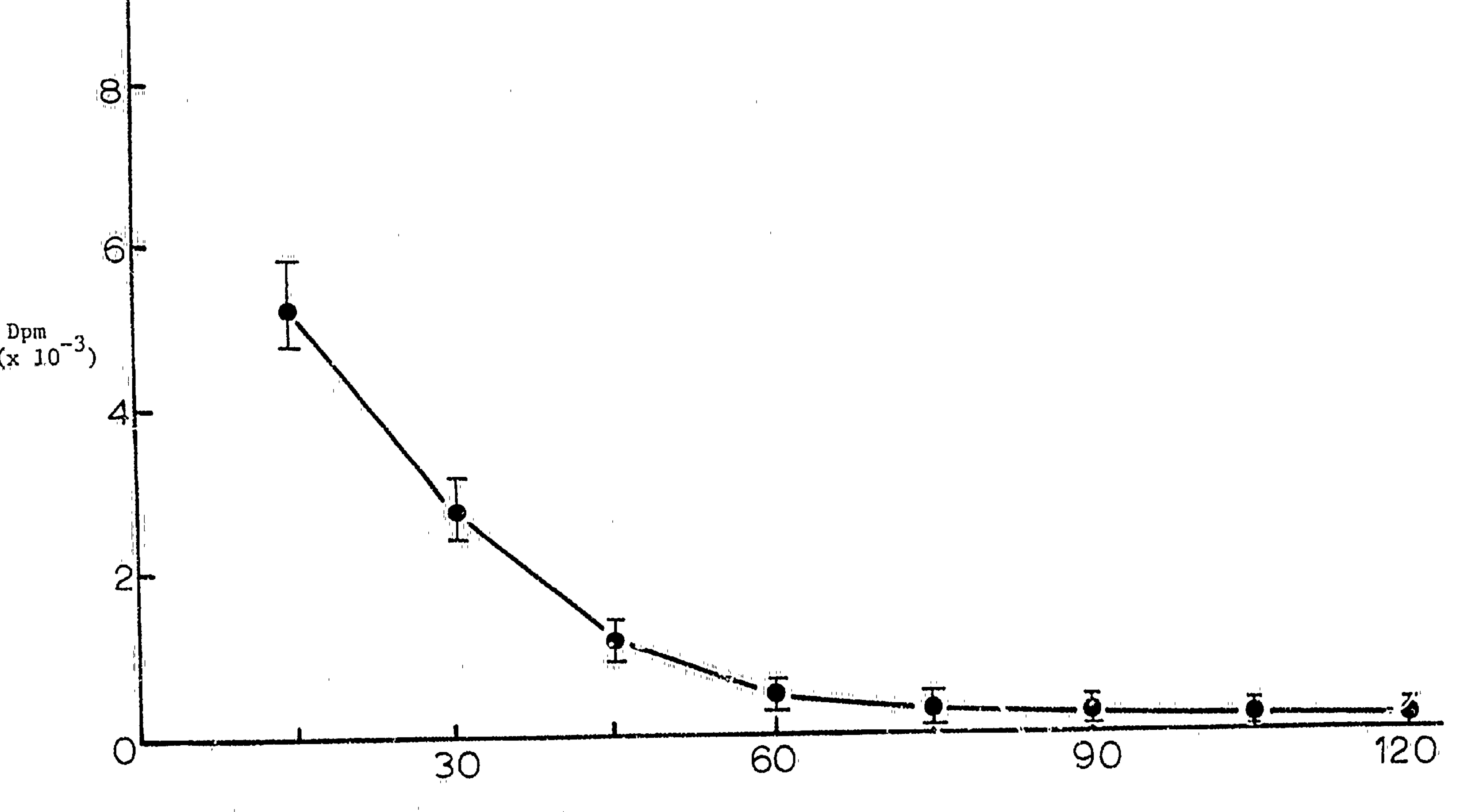
Expiration of ^{14}C-carbon dioxide after treatment with (^{14}C-COOH)-3-carboxysalsolinol (107 μmoles/Kg)

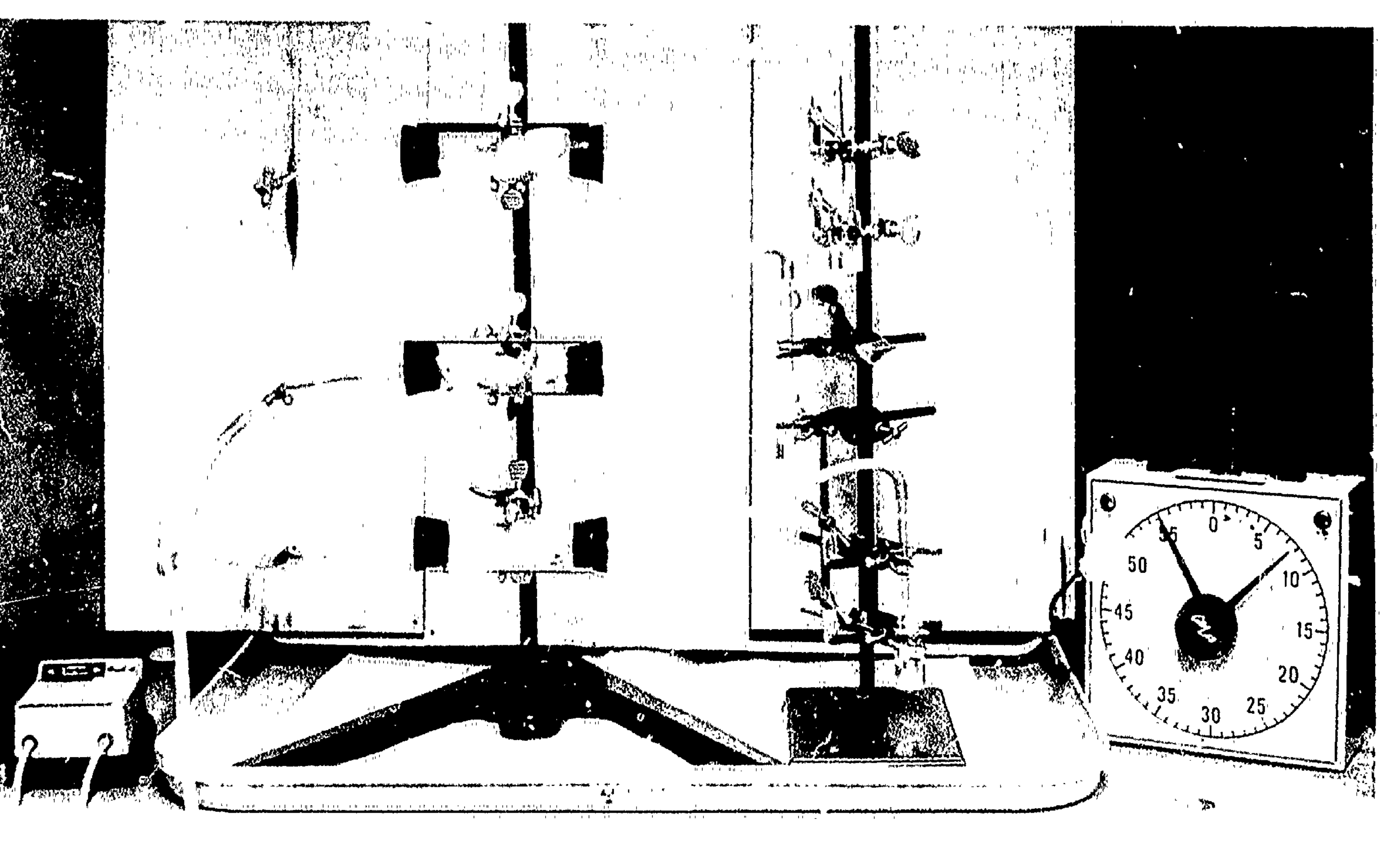
^{14}C-Carbon dioxide expired by mice after administration of (^{14}C-COOH)-3-carboxysalsolinol was collected in 15-min samples. The results were compared with the values obtained for samples from 15-min pretest periods (137 ± 11 dpm). The values in the figure represent the actual results minus 148 dpm to correct for the background count.

n = 3

counting efficiency = 73%

all values sig. diff. from background ($p < 0.05$)


Dpm
(x 10^-3)
0
2
4
6
8
30
60
90
120
Time at end of 15-minute sampling (min)



A

The results obtained following (1-^{14}C)-DOPA injections into mice are shown in Fig. 22. For three of the four mice, radioactivity readings declined in a manner similar to that observed for radiolabelled 3-carboxysalsolinol. For the entire duration of the test period, the ^{14}C-carbon dioxide emission from all three of these (1-^{14}C)-DOPA-treated mice remained significantly above the background count. This figure also shows ^{14}C-carbon dioxide emissions from a mouse that differ in time pattern with the other responses. The generated curve is biphasic with a peak at 30 min. At all times the collected levels of radioactivity exceeded background.

After a solution of (^{14}C-COOH)-3-carboxysalsolinol of lower specific activity was injected, ^{14}C-carbon dioxide was generated as shown in Fig. 23. All radioactivity readings were significantly greater than the background count. When injections of the (^{14}C-COOH)-3-carboxysalsolinol solution with lower specific activity were preceded 1 hr by the administration of the peripheral decarboxylase inhibitor carbidopa, ^{14}C-carbon dioxide was expired as illustrated in Fig. 24. The initial sample showed a radioactivity reading significantly lower than that recorded during the same interval in the absence of carbidopa (see Fig. 23). As this experiment proceeded the expiration of ^{14}C-carbon dioxide increased. The radioactivity counts of all of the samples were significantly above background levels.

Some mice received carbidopa 1 hr before treatment with (1-^{14}C)-DOPA and the results of these ^{14}C-carbon dioxide emission

Figure 22

Expiration of ^{14}C-carbon dioxide after treatment with
(1-^{14}C)-DOPA (120 μmoles/Kg)

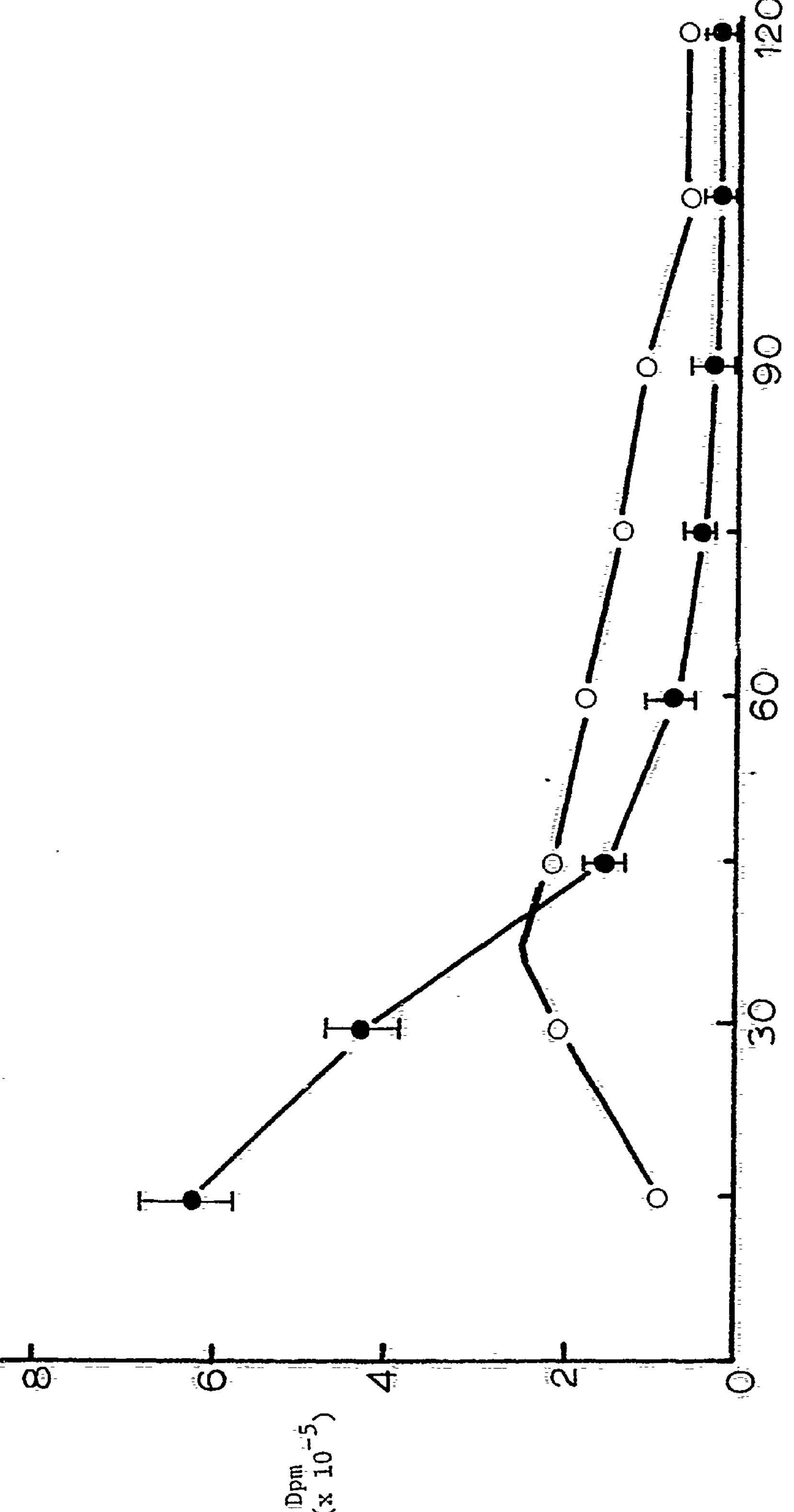
^{14}C-Carbon dioxide expired by mice after administration of (1-^{14}C)-DOPA was collected in 15-min samples. The results were compared with the values obtained for samples from 15-min pretest periods (137 ± 11 dpm).

●— n = 3

○— n = 1

counting efficiency = 73%

all values sign. diff. from background ($P < 0.001$)



Dpm
(x 10^-5)
0
2
4
6
8
30
60
90
120
Time at end of 15-minute sampling (min)

Figure 23

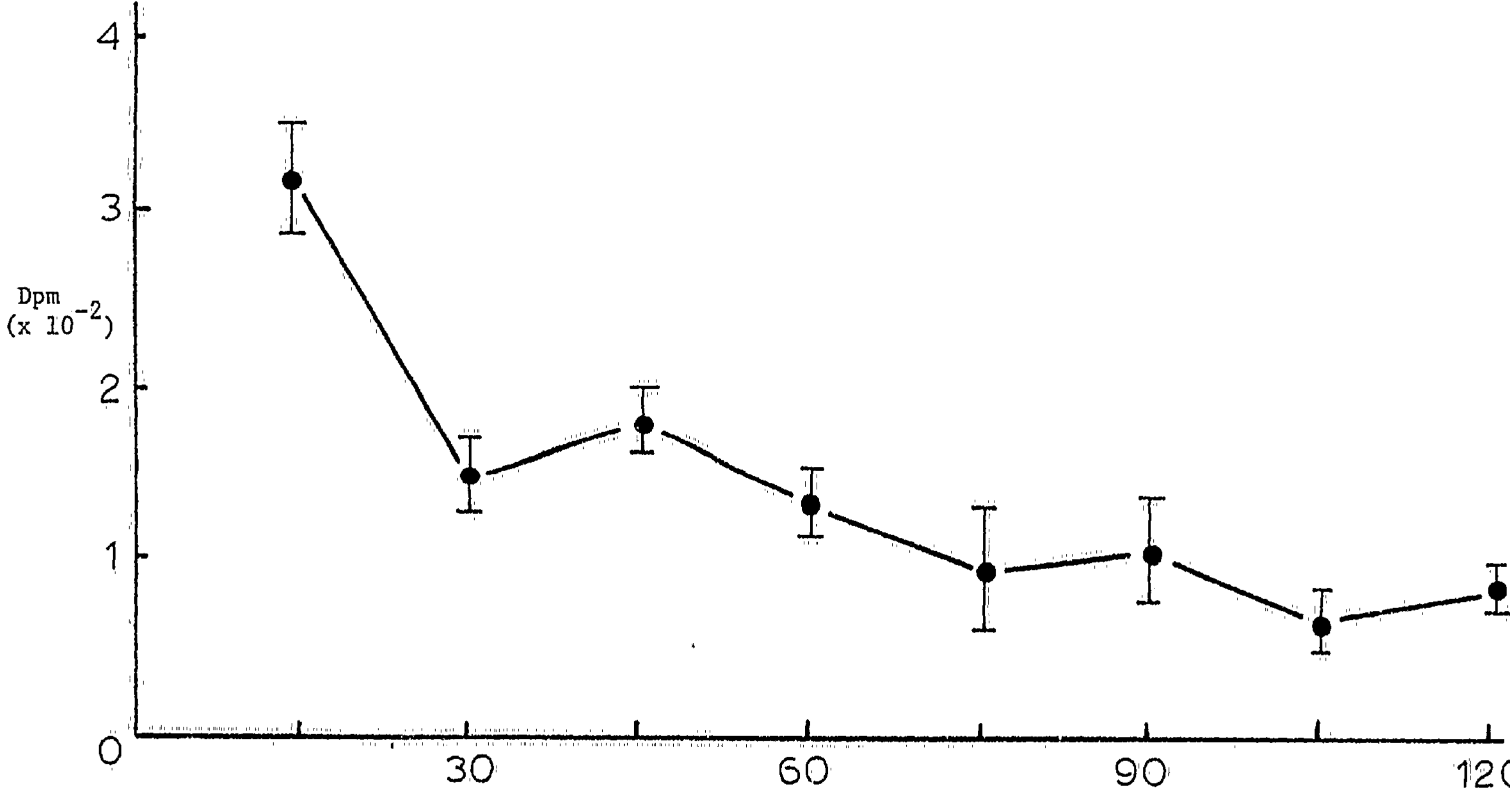
Expiration of ^{14}C-carbon dioxide after treatment with (^{14}C-COOH)-3-carboxysalsolinol (745 µmoles/Kg)

^{14}C-Carbon dioxide expired by mice after administration of (^{14}C-COOH)-3-carboxysalsolinol was collected in 15-min samples. The results were compared with the values obtained for samples from 15-min pretest periods (137 ± 11 dpm). The values in the figure represent the actual results minus 148 dpm to correct for the background count.

$n = 3$

counting efficiency = 73%

all values sig. diff. from background ($P < 0.05$)



4
3
2
1
0
Dpm
(x 10^{-2})
30
60
90
120
Time at end of 15-minute sampling (min)

Figure 24

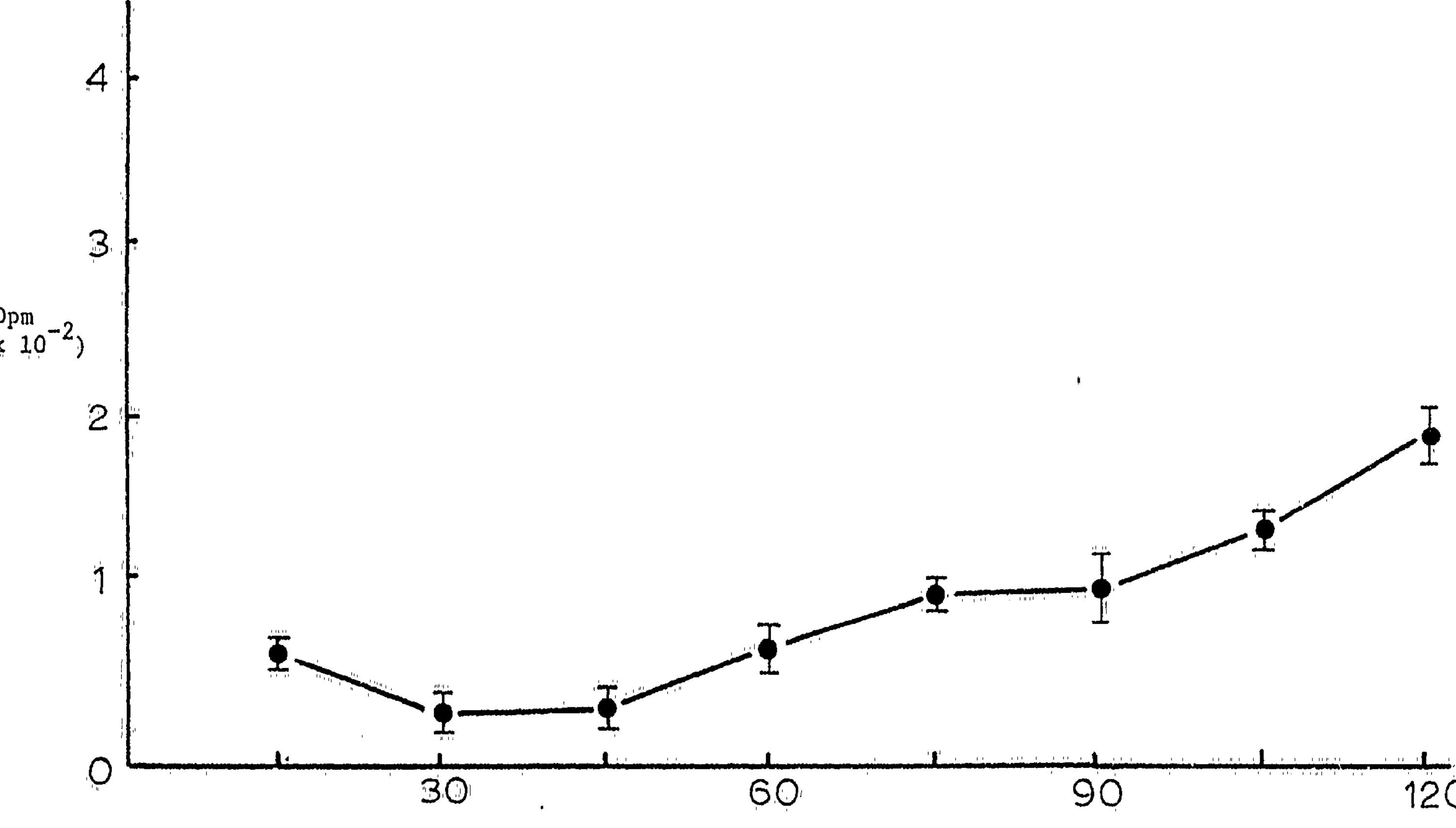
Expiration of ^{14}C-carbon dioxide after treatment with (^{14}C-COOH)-3-carboxysalsolinol (745 μmoles/Kg) and carbidopa

^{14}C-Carbon dioxide expired by mice after administration of (^{14}C-COOH)-3-carboxysalsolinol was collected in 15-min samples. Carbidopa (102 μmoles/Kg) was administered orally 1 hr before the injection of the radioisotopic TIQ. The results were compared with the values obtained for samples from 15-min pretest periods (137 ± 11 dpm). The values in the figure represent the actual results minus 148 dpm to correct for the background count.

n = 3

counting efficiency = 73%

all values sig. diff. from background ($P < 0.05$)



Dpm
(x 10^{-2})
4
3
2
1
0
30
60
90
120
Time at end of 15-minute sampling (min)

tests are illustrated in Fig. 25. Over the 2 hr test period the production of ^{14}C-carbon dioxide remained well above the background level. The maximum response, occurring between 30 and 45 min, was approximately only $\frac{1}{10}$ of the value of the maximum response observed for (1-^{14}C)-DOPA without carbidopa (see Fig. 22).

Table 5 lists the 2 hr percentage conversions of the radioisotopic 3-carboxysalsolinol and DOPA. Administration of the more radioactive solution of (^{14}C-COOH)-3-carboxysalsolinol resulted in an approximately 0.76% conversion to ^{14}C-carbon dioxide in 2 hr. With the less radioactive solution of (^{14}C-COOH)-3-carboxysalsolinol the conversion approximated 0.37% of the administered dose. Within the 2 hr period, carbidopa pretreatment did not lower significantly the conversion of the less radioactive solution of the isoquinoline. From Fig. 22, it is apparent that the test was terminated on the rising slope of the curve, so it is probable that more ^{14}C-carbon dioxide could have been produced if the study had continued. For (1-^{14}C)-DOPA, 38.3% of the administered dose was converted to ^{14}C-carbon dioxide in 2 hr. In the presence of carbidopa the decarboxylation decreased to approximately $\frac{1}{4}$ of the value obtained without carbidopa. The (1-^{14}C)-DOPA conversions were significantly greater than the conversion of (^{14}C-COOH)-3-carboxysalsolinol (107 or 745 μmoles/Kg) in the presence or absence of carbidopa.

b. Behavioural Changes Noted in the Radioactive Studies

Mice that had received radiolabelled drugs were observed for behavioural changes. During the first 30 min or so, those

Figure 25

Expiration of ^{14}C-carbon dioxide after treatment with $(1\text{-}^{14}C)$-DOPA (120 μmoles/Kg) and carbidopa

^{14}C-Carbon dioxide expired by mice after administration of $(1\text{-}^{14}C)$-DOPA was collected by 15-min samples. Carbidopa (102 μmoles/Kg) was administered orally 1 hr before the injection of $(1\text{-}^{14}C)$-DOPA. The results were compared with the values obtained for samples from 15-min pretest periods (137 ± 11 dpm).

n = 3

counting efficiency = 73%

all values are sig. diff. from background ($P < 0.001$)

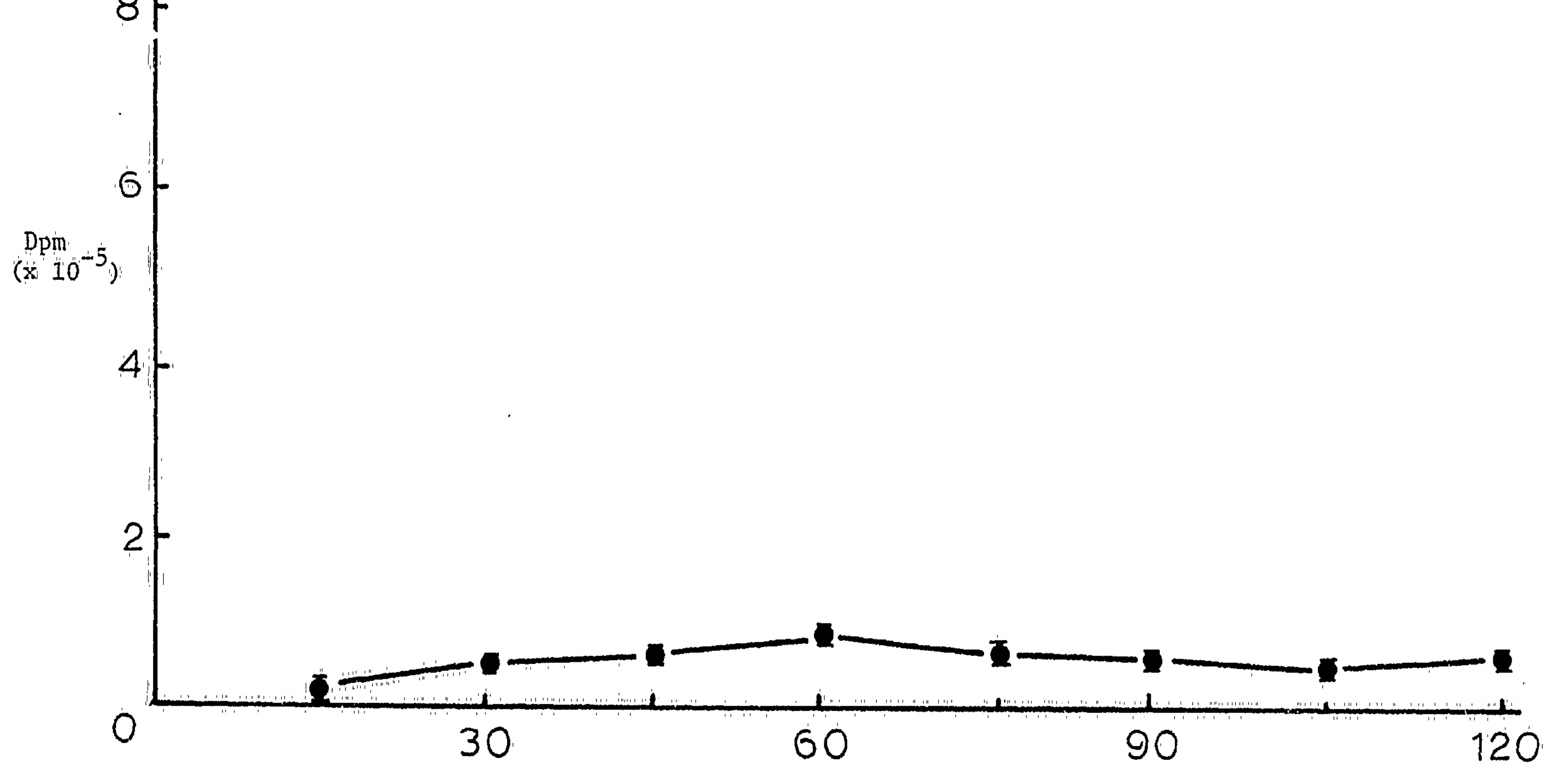

Dpm
(x 10⁻⁵)
8
6
4
2
0
30
60
90
120
Time at end of 15-minute sampling (min)

Table 5

Decarboxylation of (^{14}C-COOH)-3-carboxysalsolinol and (1-^{14}C)-DOPA *in vivo*

Drug Treatment (μ moles/Kg)	No. of dpm Injected	No. of dpm Generated	Percentage Conversion±S.E.
(^{14}C-COOH) - 3cS (107)	1.40×10^6 1.42×10^6 1.41×10^6	1.09×10^4 1.12×10^4 1.00×10^4	0.76 ± 0.025
(^{14}C-COOH) - 3cS (745)	3.42×10^5 3.22×10^5 2.86×10^5	1.54×10^3 1.19×10^3 0.83×10^3	0.37 ± 0.046
(^{14}C-COOH) - 3cS (745) + Carbidopa (102)	4.73×10^5 3.96×10^5 4.63×10^5	1.39×10^3 1.25×10^3 1.63×10^3	0.32 ± 0.017
(1-^{14}C) - DOPA (120)	3.28×10^6 3.37×10^6 3.35×10^6	1.23×10^6 1.09×10^6 1.51×10^6	38.30 ± 3.702
(1-^{14}C) - DOPA (120) + Carbidopa (102)	3.50×10^6 3.52×10^6	4.82×10^5 2.16×10^5	9.96 ± 3.812

3cS 3-Carboxysalsolinol

counting efficiency = 73%

subjects administered (^{14}C-COOH)-3-carboxysalsolinol (107 μmoles/Kg) or (1-^{14}C)-DOPA (120 μmoles/Kg) moved sporadically in the plastic tubes, sniffed the walls of the tubes, or gnawed the rubber stoppers. For the remaining time, the mice were stationary, and their eyes were closed, although somewhat jerky repositioning occurred every few minutes. By the end of the 2 hr session, the mice began to urinate and defecate. Upon being returned to their colony cage the mice explored briefly and subsequently huddled together in a quite, inactive state. These displayed behaviours were indistinguishable from those of mice injected with acidic saline or not injected at all prior to being placed in the apparatus.

Upon injection of (^{14}C-COOH)-3-carboxysalsolinol (745 μmoles/Kg) the mice remained motionless in their respective experimental chambers. Approximately once a minute the mice would reposition themselves shakily. They did not gnaw at the stoppers. Sometimes the mice would stretch out low in their tubes. The tubes remained without signs of urination or defecation. Piloerection was evident in the mice. Jerky movements disappeared after approximately the first hour. Mice would then remain stationary, with eyes closed, for at least 5 min at a time. When the subjects were returned to their colony cage at the termination of the test, they displayed a staggering gait as they briefly explored th· cage before settling down together.

Mice injected with (^{14}C-COOH)-3-carboxysalsolinol (745 μmoles/Kg) after pretreatment with carbidopa remained sedated during the

course of the experiment. Piloerection was noticeable. There was neither sniffing nor gnawing exhibited. No urination or defecation was apparent. Very little repositioning occurred. When returned to the colony cage, the mice were still untypically inactive. By approximately 36 hr after the end of the test each of the three mice had died.

Treatment with (1-^{14}C)-DOPA in the presence of carbidopa produced a quiet, inactive state in the mice. They displayed piloerection and some preening, but no urination or defecation and very little sniffing and gnawing. When placed in the colony cage, the mice became active. In contrast with the mice treated with carbidopa and the high dose of the isoquinoline, the mice receiving the decarboxylase inhibitor and radiolabelled DOPA did not succumb within the first two days after testing.

VI DISCUSSION

A. Introduction

Neural actions of ethanol and the involvement of neuroamines in those actions was reviewed earlier in this thesis. The evidence accumulated to date intimates that aberrant neuroamine metabolites may participate in some of the responses to ethanol.

Controversy over the involvement of TIQs in the effects of alcohol has fulminated in recent years. In what ways the results presented in this thesis either subdue or aggravate the discord are considered in the following discussion. Additionally, the behavioural and biochemical properties that were examined present for consideration some previously unexplored features of TIQ pharmacology.

B. TIQs in Behavioural Models of Central Activity

1. Introduction

Animal models of the acute and chronic effects of central nervous system depressants are many in number and broad ' the scope of properties examined (Bianchi and Franceschini, 1954; Kakihana et al., 1966; Kalant et al., 1971; Mello, 1976; Friedman and Lester, 1977). By focussing on the effects of TIQs in ethanol- and hexobarbital-induced narcoses, tail-clip analgesia and spontaneous locomotor activity, this dissertation reveals the properties of some

TIQs in rodent models employed in acute administration of the agents.

2. Narcosis Tests

a. Alteration of the Ethanol Response

Before any pharmacological search can be performed, the variables associated with the use of the experimental models must be considered. The tests conducted on the loss of righting reflex were certainly no exception to this tenet. Factors influencing ethanol-induced narcosis as produced in mice and rats have been examined by many investigators (McClearn, 1962; Kakihana et al., 1966; Messiha et al., 1974; Randall and Lester, 1974). Many of the expressed concerns regarding control of the variables were adopted as described in Methods.

In the ethanol narcosis experiments, dose-dependent increases in the severity of central nervous system depression was apparent after ethanol administration. The gamut of depressant effects, from ataxia to narcosis to death, was produced by the series of ethanol treatments. Not one of L-DOPA, dopamine, 3-carboxysalsolinol or salsolinol generated narcosis on its own, but each of them served to prolong ethanol-induced narcosis significantly. The results of the tests with L-DOPA and dopamine in combination with ethanol concur with previous findings (Rosenfeld, 1960; Blum et al., 1973a). These authors have reported also that serotonergic agents are capable of increasing the duration of ethanol-induced narcosis. Furthermore, the reductive pathway metabolites of dopamine and

serotonin have been shown to produce narcosis when given alone or enhance the narcosis produced by ethanol (Feldstein *et al.*, 1970; Blum *et al.*, 1973b). Following alcohol consumption elevations in the urinary levels of the reduced metabolites of noradrenaline and serotonin have been observed (Davis *et al.*, 1967a, 1967b). Such concentration increases may represent an opportunity for involvement of the reduced compounds in the central depressant effects of ethanol. Moreover, the reduced metabolites are themselves alcohols, a characteristic that may permit effects at some nonspecific alcohol site of action. However, the reductive pathway products cannot be implicated in ethanol action without prior consideration of a few noteworthy caveats. First of all, in ethanol narcosis tests the parent neuroamines have demonstrated greater potency in sleep prolongation than their precursors or reduced metabolites. Secondly, that the metabolism of dopamine is not directed towards increased reduction in the presence of ethanol and that dopamine is a potent agent in ethanol narcosis enhancement suggest the inclusion of additional factors, perhaps the formation of other classes of aberrant metabolites. The results of the experiments with the TIQs in combination with ethanol indicate that 3-carboxysalsolinol and salsolinol are more effective than the reduced metabolites of dopamine or serotonin in increasing the duration of ethanol-induced narcosis. In light of this evidence dopamine-based TIQs may be the aberrant metabolites implicated in the mechanism that prolongs the central depression produced by ethanol. Injections of L-DOPA or dopamine, along with ethanol, present to the body the substances that

condense readily to form TIQs (Robbins, 1968b; Kenyhercz and Kissinger, 1978), assuming that ADH functions to create acetaldehyde. Salsolinol and the serotonergic reduced products, the tryptophols, have generated narcosis in the absence of ethanol (Feldstein et al., 1970; Church et al., 1976). However, because the murine strain variations in sensitivity to ethanol parallel the strain responses to salsolinol, features common to both salsolinol and ethanol depressant activity appear to operate in the narcosis mechanism (Church et al., 1976).

The results indicate that in a dose of 60 μmoles/Kg 3-carboxysalsolinol given peripherally was more potent than dopamine, which in turn was more potent than L-DOPA or salsolinol, with respect to potentiating ethanol-induced narcosis. The possibility that there are differences in pharmacokinetic parameters among the four compounds, differences that may have contributed to the findings, was not examined.

Both isoquinolines demonstrated biphasic dose-response alterations of ethanol narcosis, suggesting that perhaps different doses of the isoquinolines or their metabolites liberate or otherwise interfere with the activities of different central neuroamines. That an O-methylated metabolite was involved in the responses was not investigated. However, dimethoxysalsolinol produced more of a stimulant effect in a higher dose than in a lower dose. The carboxylated TIQ manifested a 15-fold greater potency as compared with its noncarboxylated analogue. At the root of the discrepancy may be a corresponding difference in the ability of the compounds to

penetrate the blood-brain barrier. It is known that dopamine does not cross the barrier as easily as its carboxylated precursor L-DOPA (Pletscher and Gey, 1962). It is possible that a similar situation occurs for salsolinol as compared with its amino acid analogue. Upon reaching central sites the carboxylated isoquinoline may then be in a position opportune for interaction with ethanol. Alternatively, central decarboxylation to salsolinol may be the requisite reaction in the involvement of the isoquinolines in ethanol narcosis. Evidence for the decarboxylation of 3-carboxysalsolinol _in vitro_ and _in vivo_ will be discussed later.

Enzyme inhibitors were employed to amplify the hypothesis that formation of TIQs is at least in part responsible for the potentiation of ethanol narcosis observed with the biogenic amines. Pyrazole is a competitive inhibitor of ADH, and in the doses employed it suppresses the degradation of ethanol (Goldstein and Pal, 1971). This inhibitor significantly prolonged the duration of narcosis produced by ethanol alone. When 3-carboxysalsolinol was given in addition to pyrazole and ethanol, the narcosis was protracted even further. The simplest interpretation of this result would presume an interaction of the isoquinoline with elevated levels in ethanol arising from pyrazole and ethanol administration. With acetaldehyde production from ethanol impaired by pyrazole treatment, reduced quantities of acetaldehyde would be available to promote the formation either of reduced metabolites of biogenic amines or, indeed, of isoquinolines. Lack of significant enhancement of the pyrazole-ethanol narcosis responses by L-DOPA or dopamine may

reflect lack of formation of TIQs from these biogenic amines with acetaldehyde. The attenuation of the pyrazole-ethanol response by salsolinol may suggest that once again effects of this TIQ in the descending phase of the biphasic response pattern of salsolinol-ethanol narcosis were occuring. Such an explanation would require that elimination salsolinol be reduced. Also, it must be kept in mind that pyrazole has been observed to produce behavioural disruptions, such as decreases in motor co-ordination (Goldberg et al., 1972; Rydberg and Neri, 1972; MacDonald et al., 1975), and these actions may be participating in the pyrazole-ethanol narcosis tests.

Disulfiram pretreatment was employed in some tests. Its ability to inhibit AldDH and DBH has been described previously. The duration of ethanol-induced narcosis was prolonged by disulfiram. The response was further enhanced by L-DOPA. The observed prolongations may have arisen from disulfiram-induced increases in acetaldehyde, as AldDH inhibition implies less oxidation of the aldehyde. Depressant activity of acetaldehyde itself or a TIQ product of aceltadehyde and L-DOPA or one of its amine metabolites may have produced the observed effects. Furthermore, inhibition of DBH has been shown to prolong ethanol-induced narcosis (Hidaka et al., 1974). Elevations in the concentration of acetaldehyde and dopamine would be promoted by the inhibition of AldDH and DBH, respectively, by disulfiram administration. In this way, the components essential to formation of salsolinol are presented. The biphasicity of the dose-response pattern for salsolinol is again evident in these tests, with the disulfiram-salsolinol-ethanol combination of treatments generating a shorter

narcosis than salsolinol and ethanol or disulfiram and ethanol. Whether salsolinol can act as a substrate of DBH has not been examined. However, if this interaction can occur, then inhibition of DBH by disulfiram would prevent salsolinol from being converted by the enzyme and would direct it along other metabolic pathways, such as O-methylation (Collins et al., 1973). An increase by this mechanism in the concentration of the O-methylated product of salsolinol may have been responsible for the attenuation of the narcosis prolongation by salsolinol(vide supra).

It should be considered also that AldDH inhibition hinders the formation of oxidized metabolites of noradrenaline and serotonin (Davis et al., 1967a, 1967b), thereby promoting conversions along reductive pathways. Therefore, the responses observed in the presence of disulfiram may, in part, arise from the action of reduced metabolites of biogenic amines.

The MAO inhibitor pargyline produced dose-dependent increases in ethanol-induced narcosis. The responses were not affected by treatment with L-DOPA, dopamine or the isoquinolines. Interpretation of the results necessitates consideration of the following factors.

Pargyline-induced inhibition of MAO implies that preservation of monoamines is encouraged. In addition, pargyline has demonstrated the ability to increase significantly the blood acetaldehyde concentration after ethanol administration (Cohen et al., 1975). Elevation of the acetaldehyde level may have been involved in the pargyline-induced reduction in ethanol preference observed in mice

(Sanders et al., 1976). As hypothesized for disulfiram, the observed narcosis responses may have involved formation of TIQs that were demonstrated to prolong ethanol-induced narcosis. By increasing the levels of neuroamines and acetaldehyde, pargyline would have generated the components essential to the production of TIQs. That prolongations of the pargyline-ethanol response were not recorded for the amines or isoquinolines may simply reflect central concentrations of the TIQs in excess of the ones prod·cing depressant effects.

As the pargyline-ethanol response was not altered by cotreatment with L-DOPA or related agents, perhaps condensates other than TIQs can also exert effects on ethanol narcosis. Serotonin is the neuroamine suggested to have a fundamental role in sleep mechanisms (Jouvet, 1969), and it can condense with acetaldehyde to form pharmacologically active beta-carbolines, as reviewed recently (Myers, 1978). Inhibition of MAO by pargyline would prevent oxidation of serotonin, thereby permitting a greater availability of this amine for involvement in TBC formation.

Parkinson's disease with its attendant striatal deficiency of dopamine is often ameliorated by L-DOPA therapy (Cotzias et al., 1973; Hornykiewics, 1973). However, because of the profound peripheral decarboxylation of L-DOPA, necessitating high doses of the drug, incorporation of carbidopa into the treatment regimen has been attempted. Combining carbidopa and L-DOPA has resulted in a diminution of the dosage of L-DOPA required for successful therapy and an attenuation of the side-effects that accompany large doses of L-DOPA

(De la Torre, 1973; Fermaglich, 1974; Sweet et al., 1975). Carbidopa has demonstrated the ability to reduce peripheral noradrenaline synthesis without affecting central catecholamine levels, an action suggesting possible usefulness of the drug in hypertension (Wurtman and Watkins, 1977). In the narcosis tests with carbidopa and ethanol, L-DOPA prolonged narcosis significantly. This result alludes to the involvement of L-DOPA itself or a metabolite in a mechanism of central depression. Although the benserazide-ethanol response was prolonged significantly by L-DOPA, the effect was less than that observed in the carbidopa test. The hypothesis that the central depressant activity of L-DOPA arose from the conversion of this agent to dopamine and then to a decarboxylated TIQ, salsolinol, is not supported by this finding. It was expected that L-DOPA would have had no enhancing effect on the benserazide-ethanol response. That it did prolong the response may have resulted from some level of decarboxylation of L-DOPA to dopamine, with subsequent salsolinol formation and central activity. Alternatively, L-DOPA not decarboxylated in the periphery may have condensed with acetaldehyde to form 3-carboxysalsolinol, which previously displayed central depressant activity in the narcosis tests when administered peripherally.

Experiments were conducted with the isoquinolines and ethanol in the presence of decarboxylase inhibitors. The carbidopa-ethanol response was increased significantly by 3-carboxysalsolinol, while salsolinol produced no alteration of the response. The carboxyl isoquinoline attenuated the benserazide-ethanol response. The results suggest that 3-carboxysalsolinol itself, rather than its

putative decarboxylated metabolite salsolinol, is responsible for the major portion of the effects of the isoquinoline on ethanol-induced narcosis. As demonstrated in the biochemical tests (discussed later in this section), 3-carboxysalsolinol is a poorer substrate than L-DOPA for decarboxylase. Benserazide inhibition of decarboxylation may have diverted the carboxyl TIQ along the pathway leading to the production of a nondecarboxylated metabolite with central activity. O-methylation, which has been demonstrated for salsolinol, may be an important step in 3-carboxysalsolinol metabolism, and be associated with the formation of a centrally active isoquinoline (vide supra). In the ethanol narcosis tests with carbidopa and benserazide the inhibitory effect of these decarboxylase inhibitors on COMT activity (Baldessarini, 1972) was probably not a factor at the doses employed.

The results of the narcosis tests with the enzyme inhibitors suggest that isoquinolines participate in the potentiation of ethanol narcosis by L-DOPA. Although explanations have been offered for the inconsistencies presented, proof of the explanations necessitates subjecting the hypothesis to further study.

The studies on ethanol-induced narcosis indicate that the mechanisms involved in narcosis generation require further investigation. Effects of ethanol on neural membranes and on intracellular neuronal activity, basic features of central functioning, deserve more attention than has been accorded so far. The myriad of effects on neurotransmitter activity has yet to be delineated clearly. From the studies presented in this thesis it is evident that dopamine-based

TIQs prolong ethanol-induced narcosis. As biphasic rather than linear patterns of response were generated, it may be inferred that the TIQs do not produce the enhancement by a simple interference with neuronal membrane lipoproteins. The similarities of the responses to those of L-DOPA and dopamine suggest neurotransmitter involvement in the narcosis. That haloperidol did not prolong the duration of ethanol narcosis indicates that immediate alterations in dopamine receptor activity is not involved in ethanol-induced narcosis.

The preceding sets of experiments focussed on the possibility of TIQs in L-DOPA- and dopamine-enhanced ethanol narcosis. Those tests incorporating enzyme inhibitors and amines generally support the hypothesis. Some equivocal results did result, and explanations for these findings have been offered. However, the complexity of actions associated with the isoquinolines and ethanol precludes simple interpretations of the observed effects. Only after the interplay of TIQs in neuronal activity is revealed will clarification of the narcosis effects be generated. That some differences between the amines and the isoquinolines appeared alludes to differences in effects on neurochemical activity. The studies on the dopamine-related parameter spontaneous locomotor activity, to be discussed shortly in detail, suggest that 3-carboxysalsolinol can act in dopaminergic processes, perhaps by releasing the transmitter. Again, the biphasic pattern of the isoquinoline responses point to activity of the compounds in the fine adjustment of neural processes.

b. Alteration of the Hexobarbital Response

Hexobarbital acts as a depressant of the central nervous system and, like ethanol, is thought to exert its initial central effect on the ascending reticular activating system (Magoun, 1958; Kalant, 1962). The mechanism by which barbiturates induce a stuporous state in rodents has been investigated at length. The influence of inherited characteristics and environmental conditions on hexobarbital-induced narcosis in mice has been detailed by Vesell (1968). The variables that he discussed were considered prior to the establishment of our experimental protocol.

Hexobarbital was administered in our tests to mice in a dose sufficiently high to produce stupor (Katz and Yaffe, 1967; Garriott et al., 1967). The duration of narcosis produced was just slightly less than that produced by ethanol in the absence of other drugs. As with the ethanol studies, it was possible to observe either prolongations or attenuations of the responses generated by the inclusions of other drug treatments.

Neither L-DOPA nor 3-carboxysalsolinol altered the duration of hexobarbital-induced narcosis in doses that produced significant increases in the ethanol tests. As before, the doses of the amine compounds used did not produce narcosis when given alone. Furthermore, whereas pretreatment with carbidopa significantly enhanced responses produced by the amino acids in conjunction with ethanol, an enhancement in similar tests with hexobarbital did not occur. Therefore, it appears that L-DOPA- and 3-carboxysalsolinol-induced prolongation of

narcosis in the presence or absence of carbidopa is confined to the depression produced by ethanol.

Whether the mechanism of action of ethanol or barbiturates involves neuroamines or their metabolites, normal or aberrant, has been debated. The interplay of neuroamines and ethanol was considered in the previous section. That neuroamines are involved in hexobarbital-induced narcosis has been investigated recently (Wahlstrom, 1971; Brus et al., 1975). Catecholaminergic, serotonergic and cholinergic agents prolonged the duration of sleep in rats. However, tests conducted with 6-hydroxydopamine or reserpine to deplete catecholamines also indicated an increase in the length of hexobarbital-induced narcosis. Apparently, the information on the role of neuroamines in the depressant actions of barbiturates is rudimentary and needs clarification. Also, in what ways activities of neuroamines differ in ethanol or barbiturate narcosis as compared with physiological sleep (Jourvet, 1969) necessitates further investigation.

If L-DOPA prolonged ethanol-induced narcosis by means of forming an isoquinoline capable of interacting w th ethanol, then the lack of response with L-DOPA in the barbiturate test is understandable. No ethanol is present in the barbiturate tests to metabolize to the requisite condensation factor, acetaldehyde. Although the isoquinoline exerts central depressant effects on its own, as demonstrated in other sections of this report, the effects produced by 3-carboxysalsolinol and hexobarbital are probably mediated by means of dissimilar mechanisms and neural systems. Mice experiencing ethanol narcosis

demonstrated fewer body and leg jerks than did those in hexobarbital narcosis, possibly indicating different degrees of involvement of motor activity centres.

3. Analgesia Tests

Although pain and treatments that produce analgesia have been investigated at length, the central mechanisms of analgesia remain ill defined (Dykes, 1975; Mayer and Price, 1976). In rodents, several methods have been used in attempts to clarify analgesia mechanisms, and comparisons of the techniques have been reported (Winter, 1965; Brands _et al._, 1976). One sensitive method is the tail-clip technique (Haffner, 1929; Bianchi and Franceschini, 1954; Brands _et al._, 1976). Furthermore, as Bianchi and Franceschini (1954) indicated, this technique invokes central neural activity, for the mice must locate the site at which the noxious stimulus has been applied and subsequently co-ordinate their movements in order to remove it.

The results of the tests on the righting reflex suggested examination of other possible central effects of TIQs. Ross has shown that ethanol, salsolinol and opiates produced central effects on calcium depletion and binding by similar opiate-like mechanisms (Ross _et al._, 1974; Ross, 1978). TIQ analgesic activity and alteration of morphine analgesia were examined. As 3-carboxysalsolinol was the most potent isoquinoline in the ethanol narcosis tests, this congener of salsolinol was employed in analgesia investigations. As in the other studies, the responses generated by 3-carboxysalsolinol were

compared with those produced by its noncyclized progenitor L-DOPA. As noted in the Results section, 3-carboxysalsolinol produced analgesia and, as before a dose-dependent biphasic pattern of responses was observed. The pattern shifted to lower doses of 3-carboxysalsolinol in the presence of carbidopa. The isoquinoline was more potent than L-DOPA. Although a biphasic pattern was not demonstrated for L-DOPA in our tests, the results taken together with those of Major and Pleuvry (1971), who produced analgesia attenuation with higher doses of L-DOPA, suggest that such a pattern is a possibility. Furthermore, whereas 3-carboxysalsolinol at one dose, or at a lower dose in the presence of carbidopa, potentiated the analgesic response to morphine, L-DOPA in comparative tests exerted no effect on the morphine responses.

Incorporation of naloxone into our protocol abolished the maximal analgesia produced by any of the agents or combination of agents tested. In the original tests with naloxone, the drug was shown to antagonize acute opiate activity (Blumberg, et al., 1961). Since that time it has been demonstrated that naloxone penetrates into the brain rapidly (Ngai et al., 1976) and that it can block tolerance and dependence to morphine and related drugs while showing minimal agonistic activity (Kosterlitz and Watt, 1968; Martin, 1976; Yano and Takemori, 1977). Experiments performed with opiate agonists, partial agonists and antagonists and opioid peptides suggest that naloxone can interact with the various hypothesized classes of opiate receptors (Goldfarb and Hu, 1976; Goldstein, 1976; Martin, 1976; Lord et al., 1977; Snyder, 1977).

Evidence was presented in the experiments with naloxone and 3-carboxysalsolinol that the isoquinoline produced analgesia by a mechanism that may involve central opiate receptors. Also, the observation that carbidopa pretreatment enhanced the 3-carboxysalsolinol responses suggests that salsolinol may be involved in the effects, if this isoquinoline is produced by the central decarboxylation of 3-carboxysalsolinol. As mentioned above, opiate-like effects have been reported for salsolinol *in vitro*. In addition, recent work has shown that salsolinol behaves as a weak agonist-antagonist in a field-stimulated preparation of guinea-pig ileum (Hamilton *et al.*, 1976). It is possible that the biphasic pattern of analgesia produced by 3-carboxysalsolinol represented a low-dose association of the isoquinoline or a metabolite with the agonist form of the receptor and a dose-dependent gradual increase in affinity for the antagonist form (Pert and Snyder, 1974).

The administration of 3-carboxysalsolinol together with morphine produced a response significantly greater than that of morphine alone. However, the enhancement did not differ significantly from that of 3-carboxysalsolinol alone, suggesting that no 3-carboxysalsolinol-morphine interplay had occurred. Yet, the response produced by morphine and the lowest dose of 3-carboxysalsolinol in the presence of carbidopa was significantly greater than the effect generated by either agent alone in pretreated mice or by morphine alone. Interaction of morphine and 3-carboxysalsolinol or salsolinol in the same or closely-related analgesia mechanisms is suggested by this comparative enhancement. Whether or not this interaction reflects

enhanced agonist binding or an alteration in morphine metabolism or excretion is uncertain.

Dopaminergic involvement in the effects of opiates has been investigated (Lal, 1975). The acute administration of an analgesia-producing dose of morphine led to a decrease in the whole brain concentration of dopamine (Gunne _et al._, 1969; Paalzow and Paalzow, 1971). An increased rate of conversion to the acid metabolite homovanillic acid (Kuschinsky and Hornkiewicz, 1972) or to noradrenaline (Bloom _et al._, 1976) may be involved in the observed reduction in dopamine. In addition, investigations into the effects of dopaminergic agents on opiate activity have yielded valuable information. The results indicated that acute administration of dopamine agonists suppressed the acute actions of morphine, whereas treatment with dopamine receptor blockers enhanced the effects of morphine (VanderWende and Spoerlein, 1973; Eidelberg and Erspamer, 1975; Tulunay _et al._, 1976; Ferri _et al._, 1977). The dopamine-releasing action described previously for 3-carboxysalsolinol may be responsible for its increase of the analgesic response to morphine. Of considerable interest is the observation that adrenergic and dopaminergic receptor antagonists inhibit the stereospecific binding of naloxone to rat brain homogenates (Cicero _et al._, 1975; Tampier _et al._, 1977).

According to recent reports noradrenaline may be implicated in the analgesic activity of morphine. Although studies conducted with alpha-receptor blockers have yielded conflicting results (VanderWende and Spoerlein, 1973; Elliot _et al._, 1976), tests incorporating

catecholamine synthesis inhibition have proved valuable. After treatment with alpha-methyl-p-tyrosine to reduce neuronal noradrenaline quantities, morphine-induced analgesia was potentiated (Paalzow and Paalzow, 1971; Buxbaum _et al_., 1973). A similar mechanism can be projected for 3-carboxysalsolinol. If this isoquinoline is decarboxylated _in vivo_, the generated salsolinol could also free noradrenaline from its endogenous storage sites and prevent its reuptake into them, with the isoquinoline being deposited into the same sites. Such actions have been reported for salsolinol (Heikkila _et al_., 1971). The ability of 3-carboxysalsolinol itself to liberate noradrenaline neither has been proved nor refuted.

Investigations into serotonergic and cholinergic modulation of morphine-induced analgesia have yielded some definitive results (Buxbaum _et al_., 1973; Gascon and Bensemana, 1975; Tulunay _et al_., 1976). The reports indicate certain positive correlations between serotonergic and cholinergic activity and the intensity of the analgesic response to morphine.

4. Spontaneous Locomotor Activity

Investigations of drug-induced changes of locomotor behaviour in mice indicate that several factors are critical to successful experimental design. For instance, circadian influences on movement (Hutchins and Rogers, 1974) must be considered. Accordingly, the tests should be performed at times permitting the observation of drug-induced increases or decreases. Preliminary experiments in this

laboratory indicated that the strain of mice used were inactive in the morning but measureably active in the evening at the beginning of the 12-hr dark cycle. The latter situation was chosen for the drug study.

Doses of 3-carboxysalsolinol were based on those employed in the narcosis and analgesia tests. Results of the activity experiments indicated the biphasic nature of the responses. It was observed that the lowest dose employed produced an increase in spontaneous locomotor activity, whereas the highest dose produced a decrease. The intermediate dose apparently represents a transitory state, neither an overall increase nor decrease being apparent. Other investigators have reported that ethanol exerts a biphasic pattern of spontaneous locomotor activity responses in mice, with small doses augmenting and large doses attenuating the response (Svensson and Waldeck, 1973; Cott et al., 1976; Matchett and Erickson, 1977). Strain differences in the locomotor responses to ethanol have been reported. As the concentration of alcohol was increased, one strain of mice became more active and another, less active (Randall et al., 1975. The authors hypothesized separate neural mechanisms for the differing effects. As mentioned earlier, salsolinol administered intracisternally can produce strain- and dose-dependent decreases in motor activity (Church et al., 1976). The authors reported that the strain of mice more susceptible to the depressant effects of ethanol and acetaldehyde demonstrated a greater decrease in activity after salsolinol administration. The present results are complementary to the observations of Church. In addition, the low-dose increases and high dose decreases in activity observed for 3-carboxysalsolinol and reminiscent of the

biphasic effects of ethanol on locomotor activity underscore the similarity of actions of ethanol and dopamine-based TIQs.

Neuroamines have been implicated in the production of alterations in spontaneous locomotor activity. Both adrenergic and dopaminergic mechanisms have been postulated, with GABA as a mediator in the stimulant phase produced by ethanol (Cott et al., 1976). Treatment with phentolamine or alpha-methyl-p-tyrosine prevented the stimulant effect of ethanol, while propranolol antagonized the depressant effect Hutchins and Rogers, 1973; Matchett and Erickson, 1977). Administration of the dopamine-receptor antagonist spiroperidol enhanced the ethanol-induced depression of activity profoundly. In the ethanol narcosis studies with haloperidol, the lowest dose of this dopamine-receptor antagonist produced a slight prolongation of the narcosis, but the effect was not significant. However, it has also been observed that the dopamine precursor L-DOPA in the presence and absence of benserazide in doses reducing peripheral decarboxylase activity produced a decrease in activity in mice (Boismare et al., 1974). The isoquinoline THP produced a spectrum of activity effects, ranging from hyperreactivity to touch to aggressiveness to decreased motor activity at higher doses (Simon et al., 1971). THP and its metabolites have been detected in the urine of patients on L-DOPA therapy for the striatal motor disorders of Parkinsonism (Sandler et al., 1973; Coscia et al., 1977). That an isoquinoline may be involved in such malfunctions of major activity received support recently. The L-DOPA congener 3-carboxysalsolinol given peripherally decreased the striatal concentration of dopamine in mice, and the

decrease was enhanced by prior treatment with carbidopa (Hannigan and Collins, 1978).

C. Decarboxylation of DOPA and 3-Carboxysalsolinol

1. Introduction

Analyses of the behavioural experiments consistently raise the question of whether or not the various responses observed reflected actions of 3-carboxysalsolinol or its decarboxylated analogue salsolinol. Accordingly, various tests were undertaken to examine the hypothesis that 3-carboxysalsolinol could decarboxylate under experimental conditions. As before, a comparison with the noncyclic progenitor L-DOPA was made.

2. *In Vitro* Tests

Decarboxylation of L-DOPA is catalyzed by "aromatic L-amino-acid decarboxylase". The known sites and properties of the enzyme, including its lack of specificity, have been summarized recently (Iversen and Callingham, 1975). The spectrophotofluorometric assays indicated that L-DOPA was decarboxylated to dopamine by a decarboxylase homogenate prepared from guinea-pig kidney in the presence of the known cofactor pyridoxal-5'-phosphate (P5P). In the extraction process employed in the assay, the percentage recovery of dopamine was estimated to be high. Unexpectedly, the known concentrations of dopamine not introduced to resin columns generated lower fluorescence values than the resin-treated samples. Several explanations for this finding are possible. The differences could reflect an impurity in

either the dopamine sample itself or in the inorganic salts used in buffer preparations employed in the fluroescence assays of the dopamine samples not placed on the columns. Alternatively, if present in the dopamine salt itself the fluorescence inhibitor may have been removed from the dopamine sample placed on the column during the wash phases, or indeed have bound strongly to the column and not been removed during recovery of dopamine. In either case, the dopamine from the enzyme incubates was extracted on the columns and the same buffer salts were employed as in the column calibration procedure, and the calibration curve generated from resin extractions of dopamine was employed in quantitative determination of decarboxylation. As expected, L-DOPA produced dopamine, as evidenced by liberation of carbon dioxide and assays of dopamine. 3-Carboxysalsolinol gave little evidence of carbon dioxide liberation, and tests indicated that fluorescence was not produced when this substrate or salsolinol was taken through the fluorophor preparation step. It was particularly interesting that when 3-carboxysalsolinol and L-DOPA were added to the incubation medium at the same time the isoquinoline produced a dose-dependent increase in carbon dioxide liberation and dopamine formation. The mechanism responsible for this event is unknown, but several possibilities come to mind. First of all, 3-carboxysalsolinol simply may have hindered the enzymatic or nonenzymatic degradation of dopamine, giving the erroneous impression of increased formation. Secondly, the isoquinoline may have suppressed nonspecific absorption sites on the decarboxylase or other binding sites in the tissue, an action that would have directed more dopamine molecules into the

medium. It is also possible that 3-carboxysalsolinol unmasked alternate decarboxylation sites on the enzyme, thereby permitting enhanced formation of dopamine.

The cofactor P5P initially accelerates the rate of L-DOPA conversion. However, ultimately decarboxylase is inhibited by a TIQ condensate formed from L-DOPA and P5P (Schott and Clark, 1952; Sourkes, 1954; Ebadi et al., 1973). Whether only the L-DOPA-P5P TIQ is capable of interaction with decarboxylase or whether other TIQs can as well has not been explored. Perhaps by interacting with the enzyme 3-carboxysalsolinol inhibits its own decarboxylation. Another enzyme that 3-carboxysalsolinol may affect is pyridoxal kinase, which is responsible for P5P activation. TIQ compounds containing the P5P moiety inhibit the kinase, thereby limiting the amount of active P5P (Ebadi et al., 1973). If 3-carboxysalsolinol can inhibit pyridoxal kinase, dopamine formation would proceed with less suppression by P5P. Enhanced dopamine formation observed in our fluorometric tests perhaps resulted from such an occurrence. Similar explanations have been proposed by Sourkes (1954) who found that low concentrations of alpha-methyldopa potentiated the decarboxylation of the amino acid. Preliminary tests indicated that salsolinol, which yields the same native fluorescence patter as dopamine, was destroyed during fluorophor preparation and, therefore, could not have been responsible for a false dopamine reading.

In the tests on L-DOPA decarboxylation production of carbon dioxide as recorded manometrically paralleled the dopamine observations.

Such joint responses argue for genuine increases in dopamine formation. That the values recorded for carbon dioxide were smaller than those for dopamine may be a consequence of carbon dioxide dissolution into the medium.

In the enzymatic tests with radioneutral 3-carboxysalsolinol and L-DOPA the isoquinoline affected the decarboxylation of its precursor significantly, but the assay did not reveal conversion of the isoquinoline. Decarboxylation of 3-carboxysalsolinol was then examined by a more sensitive, radiochemical method by which comparative characterization of (1-^{14}C)-DOPA and (^{14}C-COOH)-3-carboxysalsolinol decarboxylation were conducted in vitro. Each of the compounds was converted enzymatically and nonenzymatically. The radiolabelled isoquinoline was decarboxylated in the presence of the enzyme homogenate at approximately 1/40th the rate of (1-^{14}C)-DOPA enzymatic conversion. In the absence of enzyme, isoquinoline conversion occurred at approximately 1/6th the rate. This difference is not due simply to an enhanced conversion of the DOPA in the presence of the enzyme for more carbon dioxide was liberated when 3-carboxysalsolinol was exposed to the enzyme than that obtained in its absence. Therefore, it would appear that 3-carboxysalsolinol can be decarboxylated by the enzyme, although it is a poor substrate. Calculations reveal that 3-carboxysalsolinol is decarboxylated enzymatically at approximately 1.7% of the rate that DOPA is converted by decarboxylase. This calculation has to be considered as a simplified result as the DOPA employed was not solely the L-isomer. In fact, some difficulty occurs in interpretation of all of the calculations, for

they are based on carbon dioxide trapped in the alkali. The efficiency of this process and the amounts of carbon dioxide remaining in the reaction media were not estimated.

Vogel (1969) has reported nonenzymatic decarboxylation of radiolabelled racemic DOPA. Various conditions of acidity and temperature altered the reaction rate. Results from the present study confirm nonenzymatic decarboxylation of DOPA and extend to incorporate nonenzymatic decarboxylation of 3-carboxysalsolinol.

3. *In Vivo* Tests

Evidence for decarboxylation of radioisotopic 3-carboxysalsolinol *in vitro* suggested an examination of the process *in vivo*. Rather than attempting to pinpoint the reaction in any one tissue it was considered more worthwhile in these initial studies to see whether the isoquinoline could be decarboxylated at all in the whole animal. For comparison, experiments were performed with radioisotopic DOPA.

Similar patterns of ^{14}C-carbon dioxide expiration were observed. In 3 hr the conversion of (1-^{14}C)-DOPA and (^{14}C-COOH)-3-carboxysalsolinol in mice was approximately 38% and less than 1%, respectively. The ratio of the conversions *in vivo* of 2.1% is of similar magnitude to the ratio (1.7%) determined *in vitro*. The aberrant pattern of ^{14}C-carbon dioxide expiration in one of the mice treated with (1-^{14}C)-DOPA was attributed to a misdirected injection. The reduced conversion could have been produced if the radiolabelled substance had been placed into a site associated with suppressed absorption.

It was considered that the conversion observed for the radioisotopic isoquinoline may have arisen from traces of unreacted radioisotopic DOPA present in the synthesized 3-carboxysalsolinol. However, a radiochemically-diluted and extensively purified sample of the isoquinoline still produced significant amounts of ^{14}C-carbon dioxide expiration.

Prior administration of carbidopa generated some unanticipated findings. Whereas treatment with the peripheral decarboxylase inhibitor suppressed ^{14}C-carbon dioxide production from (1-^{14}C)-DOPA, that from the radiolabelled isoquinoline was inhibited initially but then increased gradually. The total radioactivity produced during the first 2 hr after the administration of (^{14}C-COOH)-3-carboxysalsolinol in the presence or absence of carbidopa did not differ significantly, but the pattern of expiration within the measured intervals was greatly altered, and indicated that ^{14}C-carbon dioxide production was still in progress at the end of 2 hr. This unexpected result could have occurred if carbidopa can increase the availability of certain metal ions _in vivo_. Vogel (1969) has shown that nonenzymatic conversion of DOPA is markedly increased by ferric, ferrous and cupric ions. Alternatively, the enhanced decarboxylation may reflect penetration of 3-carboxysalsolinol through the blood-brain barrier and decarboxylation at central sites. The differences in the time patterns of the expirations observed in the presence of decarboxylase inhibition is further evidence against DOPA-trace impurities in the radiolabelled 3-carboxysalsolinol.

From the results it is not possible to discern the respective contributions of peripheral and central decarboxylation to ^{14}C-carbon dioxide production. L-DOPA is known to penetrate the blood-brain barrier (De la Torre, 1973), but no similar studies have been reported for 3-carboxysalsolinol. Nonetheless, the results of the behavioural experiments do suggest penetration of 3-carboxysalsolinol into brain areas associated with mechanisms of central activity.

Information on central and peripheral decarboxylation and the barrier between the two sites was generated in the studies with carbidopa and the radiolabelled substances. Carbidopa administration permits higher levels of L-DOPA to be achieved in the plasma (Bianchine et al., 1973) and in the brain (Lotti and Porter, 1970). Unlike the inhibitor benserazide, carbidopa does not penetrate the blood-brain barrier easily (Porter et al., 1962; Lotti and Porter, 1970). The reduction in ^{14}C-carbon dioxide expiration after (1-^{14}C)-DOPA by carbidopa pretreatment suggests that the gas was produced mainly in peripheral sites. That the expiration was not abolished by carbidopa indicates that the enzyme was not totally inhibited peripherally, that some central decarboxylation was involved, that nonenzymatic conversion occurred, or that a combination of these events was responsible.

Investigations into the decarboxylation of 3-carboxysalsolinol do suggest the production of salsolinol *in vivo*. Therefore, the biochemical data support the possibility that salsolinol participates in behavioural results with 3-carboxysalsolinol. The only deaths

observed following administration of the carboxyl isoquinoline were those accompanying high doses of 3-carboxysalsolinol given in the presence of carbidopa. The manner of death suggested central depression as a basic mechanism. Hjort *et al*. (1942) has shown an LD_{50} of 417 mg/Kg for salsolinol in mice, and the deaths occurred without terminal convulsions. In contrast, the 6- and 7-methoxy congeners of salsolinol produced signs of stikulation, such as tremors and convulsions (Hjort *et al*., 1942 Teitel *et al*., 1974). The latter findings are in accord with the stimulatory effect of dimethoxysalsolinol observed in the narcosis tests. Church *et al*. (1976) has shown central depressant activity for salsolinol, and the study involving 3-carboxysalsolinol in spontaneous locomotor activity has shown that the carboxyl TIQ can produce central depression. In light of the evidence that carbidopa progressively enhances carbon dioxide expiration after 3-carboxysalsolinol, and presuming that this expiration is paralleled by salsolinol formation, the observed deaths could be due to the production of toxic quantities of salsolinol.

D. The Role of TIQs in the Actions of Ethanol and Opiates

While this study was in progress several significant actions of TIQs were revealed. For instance, Myers and Melchior (1977b) showed dramatic increases in alcohol consumption after intraventricular applications of low doses of isoquinoline compounds. Ross *et al*. (1974) demonstrated that very low doses of salsolinol can produce marked changes in ionic binding of calcium to brain synaptosomes in parallel to the actions of ethanol and opiate agonists. Recently, an isoquinoline was identified in the brains of mice exposed chronically to alcohol

(Hamilton *et al.*, 1978). These various discoveries promote an association between isoquinolines and alcoholism and suggest the possibility of an opiate link in alcohol dependence (Blum *et al.*, 1978).

The results presented in this thesis offer support for the involvement of dopamine-based isoquinolines in ethanol-related responses. Both salsolinol and 3-carboxysalsolinol can amplify the acute depressant effect of ethanol, as measured in the narcosis studies. The results from the locomotor activity study, the barbiturate narcosis investigation and the tests on analgesia suggest that the effect on ethanol narcosis is not due to a generalized central depressant action, but offer support for a relatively specific association with ethanol and, perhaps, morphine.

The mechanism for these effects is not known, but the comparative studies with the noncyclized progenitors indicate differences of sufficient magnitude to conclude that there is more involved than dopamine-based interactions. As discussed elsewhere in this thesis, isoquinoline compounds cause many actions in neurotransmitter systems. Based upon results obtained with 3-carboxysalsolinol it is proposed that it can react in a number of ways in monoamine neurons in the central nervous system, including the possibility that it may be an agonist in its own right. 3-Carboxysalsolinol must be able to cross the blood-brain barrier, and subsequently may be taken up, stored and released in catecholaminergic systems. In such locations, the isoquinoline may interact with enzymes associated with neurons either to alter the state of the endogenous neuroamines or to undergo conversion itself to a product or products capable of displaying transmitter-like qualities. Salsolinol, the proposed decarboxylation

product of 3-carboxysalsolinol, may be formed intraneuronally by the action of decarboxylase. It could be stored and released and subsequently may undergo o-methylation by COMT. It is possible that the o-methylated isoquinoline serves to alter neural activity. Presynaptic or postsynaptic interactions of the dopamine-based isoquinolines, acting as either agonists or antagonists, would provoke physiologically-significant shanges, most likely in catecholaminergic systems. Undoubtedly, a complex of actions could occur and may be responsible for the effects noted in this study. Future work based upon the current findings would reveal the validity of these hypotheses.

In conclusion, it must be stated that these studies have examined only acute behavioural effects of the isoquinolines and, as such, can offer just partial support for the "link" hypothesis mentioned previously. Future work on the effects of chronic treatment with isoquinolines should help to reveal the role of these agents in the development of dependence on, and tolerance to, ethanol. The pharmacological prperties of the isoquinolines identified at this time offer some support for the suggestion that these characteristics may be expressed through actions on morphine-sensitive receptors in the central nervous system. Through continued efforts to elucidate the effects of acute and chronic isoquinoline administration, man will achieve a greater understanding of the neural actions of drugs and, perhaps, the foundations of addictive phenomena.

VII SUMMARY AND CONCLUSIONS

Through reflection on the preceding discourse the following salient features of the research emerge.

1. In a dose of 60 μmoles/Kg 3-carboxysalsolinol given peripherally was more potent than dopamine, which in turn was more potent than L-DOPA or salsolinol, with respect to prolonging ethanol-induced narcosis in mice.

2. 3-Carboxysalsolinol and salsolinol demonstrated biphasic response patterns of ethanol narcosis prolongation. Increased doses generated increased durations of narcosis, a response pattern that peaked and declined upon further dosage increases.

3. 3-Carboxysalsolinol prolonged the narcosis response to ethanol in combination with pyrazole. Salsolinol attenuated the response. The results demonstrated the biphasicity of the TIQ response patterns and suggest TIQ-ethanol interactions.

4. L-DOPA prolonged the duration of narcosis generated by ethanol with disulfiram. Salsolinol attenuated the response. The results suggest endogenous formation of an L-DOPA-based TIQ that has central depressant activity.

5. Increases in neuroamine and acetaldehyde concentrations after ethanol administration may function in the increases observed in the duration of ethanol narcosis after pargyline pretreatment.

6. Lack of significant effects with haloperidol intimates that dopamine receptor activations are not involved in the acute narcotic response to ethanol.

7. L-DOPA prolonged narcosis produced by ethanol with carbidopa or benserazide. Increased peripheral or central quantities of L-DOPA and acetaldehyde, which was generated from ethanol, may have condensed to form the TIQ 3-carboxysalsolinol that in turn may have prolonged the narcosis.

8. 3-Carboxysalsolinol, but not salsolinol, enhanced the narcosis response to ethanol and carbidopa, a peripheral decarboxylase inhibitor. The carboxyl TIQ decreased the response to ethanol and benserazide, an inhibitor of peripheral and central decarboxylase. Therefore, the carboxylated TIQ itself excited a greater effect on the duration of ethanol narcosis than the decarboxylated metabolite, and the decrease observed in the presence of benserazide may reflect an effect of a TIQ in the descending phase of the biphasic response pattern.

9. L-DOPA or 3-carboxysalsolinol given in the presence or absence of carbidopa exerted no effect on the duration of hexobarbital-induced narcosis. It is concluded that the narcotic effects of L-DOPA and 3-carboxysalsolinol are confined to ethanol-related narcosis mechanisms.

10. 3-Carboxysalsolinol produced analgesia in the murine tail-clip test in a dose-dependent biphasic pattern, which shifted to lower doses of the TIQ in the presence of carbidopa. The isoquinoline was more potent than L-DOPA as an analgesic.

11. The analgesic response produced by morphine and the lowest dose of 3-carboxysalsolinol in the presence of carbidopa pretreatment was significantly greater than the effect of either agent alone in pretreated mice or morphine alone. Naloxone blockade of the responses produced by the agents given individually or in combination suggest closely-related analgesia mechanisms for 3-carboxysalsolinol and morphine and to a lesser extent, L-DOPA. The biphasic pattern evinced by 3-carboxysalsolinol may represent either a low-dose association of the TIQ with the agonist form of an opiate receptor and a dose-dependent gradual increase in affinity for the antagonist form or the invocation of an endogenous opiate mechanism.

12. 3-Carboxysalsolinol produced a biphasic low-dose increase and high-dose decrease in spontaneous locomotor activity in mice, mimicking the pattern displayed by ethanol as recorded by other authors. The results suggest involvement of the TIQ in the dopaminergic motor activity mechanisms.

13. Decarboxylation of L-DOPA to dopamine and carbon dioxide was enhanced by 3-carboxysalsolinol _in vitro_. Interaction of the TIQ with amino-acid decarboxylase would present a possible explanation for the observed responses.

14. (^{14}C-COOH)-3-Carboxysalsolinol and (1-^{14}C)-DOPA were decarboxylated nonenzymatically and enzymatically *in vitro*, as determined by ^{14}C-carbon dioxide measurements. The radiolabelled TIQ decarboxylated in the presence of the enzyme homogenate at approximately 1/40th the rate that (1-^{14}C)-DOPA was converted. Without the homogenate, conversion occurred at approximately 1/6th the rate. Nonenzymatic decarboxylation may be of greater importance to 3-carboxysalsolinol conversion than to its amino-acid precursor.

15. (^{14}C-COOH)-3-Carboxysalsolinol and (1-^{14}C)-DOPA were decarboxylated *in vivo*, at rates representing less than 1% and less than 39%, respectively. Carbidopa pretreatment attenuated the response to radiolabelled DOPA and, initially, to radiolabelled 3-carboxysalsolinol. However, decarboxylation of radiolabelled DOPA was slightly enhanced during the second hour of the test. That of the radiolabelled TIQ was profoundly increased in the terminal half of the test. Decarboxylation centrally or peripherally, nonenzymatically and enzymatically by other decarboxylases, may be responsible for the results recorded.

16. In conclusion, studies conducted on the pharmacology of dopamine-based TIQs suggest that they may be invoked in the actions of ethanol and may demonstrate interactions with opiate receptor mechanisms. Moreover, salsolinol produced by the decarboxylation of 3-carboxysalsolinol may be the TIQ responsible for at least some of the central effects observed after the administration of the carboxylated isoquinoline.

VIII SUGGESTIONS FOR FUTURE RESEARCH

Any study is incomplete and suggests other avenues for exploration. Some of these directions are considered below.

The effects of pargyline and the amines in ethanol narcosis need clarification. A wider range of doses of pargyline should be employed, especially at the lower end of the scale. Such studies should serve to indicate whether the responses recorded in the present investigation with L-DOPA reflect an overshoot of the peak dose-response effect.

In the ethanol narcosis tests, dimethoxysalsolinol exerted a stimulant effect. It would be of interest to determine whether, as for 3-carboxysalsolinol, the effect is confined to ethanol, or whether the agent is a stimulant in hexobarbital narcosis also.

3-Carboxysalsolinol displayed a biphasic pattern of responses in analgesia tests. In the doses employed, L-DOPA produced only an increase. It would be beneficial to employ higher doses of L-DOPA in an attempt to confirm the biphasic response of this agent in analgesia (Major and Pleuvry, 1971).

In experiments on nonenzymatic decarboxylation of DOPA, Vogel (1969) showed that inorganic ions significantly affected the rate of conversion. It would be useful to know the degree of influence of the ions on the nonenzymatic decarboxylation of 3-carboxysalsolinol.

Dopamine also increased the rate of conversion of DOPA nonenzymatically and, considering that the isoquinoline has been found to influence dopaminergic systems, similar tests with 3-carboxysalsolinol should provide useful information. The increased formation of carbon dioxide in the second hour of the carbidopa-)^{14}C-COOH)-3-carboxysalsolinol test may have resulted from such interactions.

Formation of dopamine and carbon dioxide from L-DOPA was increased by 3-carboxysalsolinol in enzymatic tests. Accordingly, the inclusion of the radioneutral isoquinoline into carbon dioxide expiration tests with (1-^{14}C)-DOPA should prove beneficial.

The development of a very sensitive assay for repeated blood ethanol and acetaldehyde measurements in individual mice would have been of immense value. Such tests would have permitted the determination of drug-induced changes in blood levels of these substances. An attempt to develop such a method using gas chromatography was not successful, but it is possible that radiotracer methods could have the inherent sensitivity required. Appropriate manipulations could perhaps, afford methods in which direct blood analysis is not essential. By means of expiration studies incorporating aldehyde and carbon dioxide trapping agents, collection and quantitation of expired ethanol and acetaldehyde may be attainable with continuous sampling from individual mice without injury to the subjects.

APPENDICES

APPENDIX 1

Enzyme inhibitors were used in some of the experiments. The chemical structures of the inhibitors pyrazole, disulfiram, pargyline, carbidopa and benserazide are illustrated in Fig. 26.

Figure 26

Chemical structures of enzyme inhibitors

1. Pyrazole
2. Disulfiram
3. Pargyline
4. Carbidopa
5. Benserazide

1. (ring structure with N, N)

2. $(H_3CH_2C)_2N-C(=S)-S-S-C(=S)-N(CH_2CH_3)_2$

3. $C_6H_5-CH_2-N(CH_3)-CH_2-C\equiv CH$

4. $(HO)_2C_6H_3-CH_2-C(CH_3)(NH-NH_2)-COOH$

5. $(HO)_3C_6H_2-CH_2-NH-NH-C(=O)-CH(NH_2)-CH_2OH$

Appendix 2

In Table 6 the baseline values for spontaneous locomotor activity are recorded for six of the test intervals. To the four groups of mice that generated the first four rows of values, acidic saline was administered on the evening subsequent to the one on which the baseline recordings were taken. All of the other mice received 3-carboxysalsolinol dissolved in the vehicle.

Table 6

Baseline spontaneous locomotor activity

3-Carboxysalsolinol (μmoles/Kg)*	Activity (counts/group of 5 mice) at 6-12	30-36	54-60	90-96	114-120 min
0	588	657	549	555	408
	666	576	555	369	564
	750	627	567	426	495
	564	402	555	429	420
38	768	456	651	441	306
	762	783	732	678	768
	834	750	537	723	765
	405	309	381	327	381
	597	648	540	537	372
	696	549	573	348	558
100	450	432	513	414	387
	783	792	663	699	804
	735	777	810	750	765
	747	600	564	414	489
190	804	789	729	687	690
	810	798	762	600	720
	885	840	732	485	462
	564	474	453	348	309
	465	492	480	447	378
	543	384	585	402	414

*Dose of drug to be injected on the following evening.

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