CARDIOVASCULAR RESPONSE TO BETA-HYDROXY THUJAPLICIN AND GAMMA-THUJAPLICIN

by

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A thesis submitted in partial fulfilment of the requirements for the degree of
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in the Division of Pharmacology
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We accept this thesis as conforming to the required standard

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Department of Pharmaceutical Sciences

The University of British Columbia
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ABSTRACT

An investigation was undertaken to determine the effects of beta-hydroxy thujaplicin and gamma-thujaplicin, used as the sodium salts, on blood pressure and heart rate in the rat. Attempts were made to discover the sites and modes of action of these two compounds.

Gamma-thujaplicin 30mg/kg. produced either a vasopressor or a vasodepressor response in anesthetized rats. The pressor response was usually more pronounced than the vasodepressor response. Tachycardia occurred with either blood pressure response. To determine whether the central nervous system was necessary for the vasopressor and tachycardiac response, pithed rats were used. In these preparations gamma-thujaplicin produced only a fall in blood pressure and a decrease in heart rate.

The effect of adrenergic blocking drugs on the response to gamma-thujaplicin was investigated. In the anesthetized rat the vasopressor response was reduced significantly by both phenoxybenzamine and pronethalol; however, the heart rate was
unaffected. In the pithed rat the vasodepressor response produced by gamma-thujaplicin was not affected by pronethalol; however, gamma-thujaplicin produced a significantly greater decrease in heart rate after treatment with pronethalol.

To determine whether gamma-thujaplicin had adrenergic alpha-receptor or beta-receptor blocking properties its effect on blood pressure responses to noradrenaline and isoproterenol was investigated. Gamma-thujaplicin was found to reduce the vasopressor effect of intravenous noradrenaline 0.5ug/kg. but had no effect on the vasopressor response produced by isoproterenol 0.25ug/kg. The pressor response to intravenous physostigmine salicylate 40ug/kg. was unaffected by gamma-thujaplicin. The increase in heart rate and blood pressure produced by gamma-thujaplicin, with injections repeated every fifteen minutes, was greatly reduced after the second dose.

It is concluded that in the anesthetized rats the vasopressor and tachycardiac response produced by gamma-thujaplicin were of central origin while the vasodepressor effect was a result of direct action on vascular smooth muscle. The vasopressor response could involve the stimulation, via sympathetic nerves, of alpha-adrenergic receptors of vascular smooth muscle. Gamma-thujaplicin can produce alpha-adrenergic receptor blockade to exogenous noradrenaline, but not to endogenously released noradrenaline since the response to intravenous physostigmine was not affected by the tropolone.
Beta-hydroxy thujaplicin 10mg/kg caused a vaso­pressor response in all anesthetized and pithed rats tested. The vasopressor response was abolished by phenoxybenzamine in both anesthetized and pithed rats. Beta-hydroxy thuja­plicin did not alter isoproterenol induced tachycardia or the vasopressor response produced by physostigmine salicylate.

It is concluded that beta-hydroxy thujaplicin caused its vasopressor effect in rats by acting directly on the alpha-adrenergic receptors of vascular smooth muscle.

Signatures of Examiners
# TABLE OF CONTENTS

<table>
<thead>
<tr>
<th>Chapter</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>ABSTRACT</td>
<td>1</td>
</tr>
<tr>
<td>LIST OF TABLES</td>
<td>vii</td>
</tr>
<tr>
<td>LIST OF FIGURES</td>
<td>viii</td>
</tr>
<tr>
<td>INTRODUCTION</td>
<td>1</td>
</tr>
<tr>
<td>LITERATURE SURVEY</td>
<td>2</td>
</tr>
<tr>
<td>Tissue Catecholamines</td>
<td>2</td>
</tr>
<tr>
<td>Distribution</td>
<td>2</td>
</tr>
<tr>
<td>Synthesis</td>
<td>2</td>
</tr>
<tr>
<td>Tyrosine Hydroxylase</td>
<td>3</td>
</tr>
<tr>
<td>DOPA-Decarboxylase</td>
<td>3</td>
</tr>
<tr>
<td>Dopamine-Beta-Hydroxylase</td>
<td>3</td>
</tr>
<tr>
<td>Inactivation</td>
<td>4</td>
</tr>
<tr>
<td>Monamine Oxidase</td>
<td>4</td>
</tr>
<tr>
<td>Catechol-O-Methyl Transferase</td>
<td>6</td>
</tr>
<tr>
<td>Uptake of Catecholamines by Sympathetic Nerves</td>
<td>7</td>
</tr>
<tr>
<td>Cardiovascular Pharmacology</td>
<td>11</td>
</tr>
<tr>
<td>Isoproterenol and Pronethalol</td>
<td>14</td>
</tr>
<tr>
<td>Noradrenaline and Phenoxybenzamine</td>
<td>16</td>
</tr>
<tr>
<td>Tropolones</td>
<td>18</td>
</tr>
<tr>
<td>Chemistry</td>
<td>18</td>
</tr>
<tr>
<td>Pharmacology</td>
<td>20</td>
</tr>
<tr>
<td>EXPERIMENTAL</td>
<td>Page</td>
</tr>
<tr>
<td>-----------------------------------------------------------------------------</td>
<td>------</td>
</tr>
<tr>
<td><strong>Method</strong></td>
<td>23</td>
</tr>
<tr>
<td>Operative Procedure</td>
<td>23</td>
</tr>
<tr>
<td>Drugs Employed</td>
<td>24</td>
</tr>
<tr>
<td>Tabulation of Heart Rate and Blood Pressure Data</td>
<td>25</td>
</tr>
<tr>
<td>Evaluation of Alpha-Receptor or Beta-Receptor Blockade</td>
<td>26</td>
</tr>
<tr>
<td><strong>Results</strong></td>
<td>26</td>
</tr>
<tr>
<td>Gamma-Thujaplicin</td>
<td>26</td>
</tr>
<tr>
<td>Effect on Blood Pressure and Heart Rate</td>
<td>26</td>
</tr>
<tr>
<td>Intact Rats</td>
<td>26</td>
</tr>
<tr>
<td>Repeated Doses</td>
<td>29</td>
</tr>
<tr>
<td>Pithed Rats</td>
<td>30</td>
</tr>
<tr>
<td>Effect of Adrenergic Blocking Drugs on the Response to Gamma-Thujaplicin</td>
<td>31</td>
</tr>
<tr>
<td>Effect of Gamma-Thujaplicin on the Responses to Noradrenaline and Isoproterenol</td>
<td>34</td>
</tr>
<tr>
<td>Effect of Gamma-Thujaplicin on the Vasopressor Response to Physostigmine</td>
<td>37</td>
</tr>
<tr>
<td>Beta-Hydroxy-Thujaplicin</td>
<td>38</td>
</tr>
<tr>
<td>Effect on Blood Pressure and Heart Rate</td>
<td>38</td>
</tr>
<tr>
<td>Anesthetized Rats</td>
<td>38</td>
</tr>
<tr>
<td>Pithed Rats</td>
<td>38</td>
</tr>
<tr>
<td>Repeated Doses</td>
<td>39</td>
</tr>
<tr>
<td>Effect of Phenoxybenzamine on the Response to Beta-Hydroxy Thujaplicin</td>
<td>40</td>
</tr>
<tr>
<td>Effect of Beta-Hydroxy Thujaplicin on Isoproterenol</td>
<td>41</td>
</tr>
<tr>
<td>Induced Tachycardia</td>
<td>41</td>
</tr>
<tr>
<td>Effect of Beta-Hydroxy Thujaplicin on the Vasopressor Response to Physostigmine</td>
<td>42</td>
</tr>
</tbody>
</table>
DISCUSSION

Gamma-Thujaplicin
Beta-Hydroxy Thujaplicin

SUMMARY AND CONCLUSIONS

BIBLIOGRAPHY
## LIST OF TABLES

<table>
<thead>
<tr>
<th>Table</th>
<th>Description</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>Effect on Blood Pressure and Heart rate of Repeated Injections of Gamma-Thujaplin 30mg/kg</td>
<td>30</td>
</tr>
<tr>
<td>II</td>
<td>The Effect of Pronethalol 10mg/kg. and Phenoxylbenzamine 5mg/kg. on the Response to Gamma-Thujaplin 30mg/kg</td>
<td>32</td>
</tr>
<tr>
<td>III</td>
<td>Effect of Gamma-Thujaplin on the Vasopressor Response to Physostigmine</td>
<td>37</td>
</tr>
<tr>
<td>IV</td>
<td>Effect on Blood Pressure of Repeated Injections of Beta-Hydroxy Thujaplin 10mg/kg</td>
<td>40</td>
</tr>
<tr>
<td>V</td>
<td>Effect of Phenoxylbenzamine 5mg/kg. on the Vasopressor Response to Beta-Hydroxy Thujaplin 10mg/kg</td>
<td>41</td>
</tr>
<tr>
<td>VI</td>
<td>Effect of Isoproterenol 0.25ug/kg. Before and After Treatment with Beta-Hydroxy Thujaplin 10mg/kg</td>
<td>42</td>
</tr>
</tbody>
</table>
# LIST OF FIGURES

<table>
<thead>
<tr>
<th>Figure</th>
<th>Description</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>Pathway for Metabolism of Noradrenaline.</td>
<td>5</td>
</tr>
<tr>
<td>2.</td>
<td>Mechanical Forces which Determine the Minute Output of Ventricles.</td>
<td>12</td>
</tr>
<tr>
<td>3.</td>
<td>Factors which Determine the Resistance to Blood Flow.</td>
<td>13</td>
</tr>
<tr>
<td>4.</td>
<td>The Two Combinations of Haloalkylamines with the Alpha-Receptor.</td>
<td>18</td>
</tr>
<tr>
<td>5.</td>
<td>Structure of Tropolones.</td>
<td>19</td>
</tr>
<tr>
<td>6.</td>
<td>The Vasopressor and Tachycardiac Response Produced by Gamma-Thujaplicin 30mg/kg. in Anesthetized Intact Rats</td>
<td>27</td>
</tr>
<tr>
<td>7.</td>
<td>The Vasodepressor and Tachycardiac Response Produced by Gamma-Thujaplicin 30mg/kg. in Anesthetized Intact Rats.</td>
<td>28</td>
</tr>
<tr>
<td>8.</td>
<td>Effect of Gamma-Thujaplicin 7mg/kg. on Responses to Noradrenaline.</td>
<td>35</td>
</tr>
<tr>
<td>9.</td>
<td>Effect of Gamma-Thujaplicin 7mg/kg. on Responses to Isoproterenol.</td>
<td>36</td>
</tr>
<tr>
<td>10.</td>
<td>The Vasopressor Effect of Beta-Hydroxy Thujaplicin 10mg/kg. in Anesthetized Intact Rats.</td>
<td>39</td>
</tr>
</tbody>
</table>
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INTRODUCTION

The natural occurring tropolones found in the western red cedar tree are alpha-thujaplicin, beta-hydroxy thujaplicin, beta-thujaplicin and gamma-thujaplicin. Limited studies have been done on these tropolones which could indicate sympathetic nervous activity. It has been found that beta-thujaplicin caused a fall in blood pressure in the guinea pig and rabbit (52). Gamma-thujaplicin produced a decrease in blood pressure and heart rate in the cat(56). In small doses beta-thujaplicin increased the height of contraction of the aortic strip of the rabbit when stimulated with adrenaline(60). Gamma-thujaplicin has a positive inotropic action on isolated atria while beta-hydroxy-thujaplicin has no effect on this preparation (personal communication, Dr. J.E. Halliday). The tropolones and catechol rings are biochemically isosteric. It would be interesting if they would display an affinity for a receptor or receptors of the sympathetic nervous system. This would be the only group of compounds known which do not have an amine and have an effect on an adrenergic receptor. The effect could be via a slightly different mechanism from that of the catecholamines and perhaps allow a deeper understanding of the structural nature of the adrenergic receptor.

This study on the cardiovascular response was undertaken in an attempt to determine whether thujaplicins 'in vivo' affect the adrenergic mechanisms.
LITERATURE SURVEY

Tissue Catecholamines

Research during the last decade has increased our knowledge of the tissue catecholamines, dopamine, noradrenaline and adrenaline, with respect to distribution, function, synthesis, storage, release and inactivation. The brief discussion which follows refers to their role in the peripheral sympathetic nervous system.

Distribution

The identification of noradrenaline as the transmitter substance in the postganglionic sympathetic nerve fibre was made by von Euler in 1946(1,2). The development of histochemical and biochemical methods made it possible to show the intraneuronal localization of the amine. It was found that the monoamines were stored in special structures in the nerve cells, called granules, which are concentrated in the nerve terminals. A large number of enlargements or varicosities of the nerve endings, each containing many granules, are localized close to the effector cells(3,4). Noradrenaline is distributed between a mobile pool in the cytoplasm and the more stable reserve form within the granules.

Synthesis

In 1938 the enzyme dihydroxyphenylalanine(DOPA) decarboxylase was discovered by Holtz, Heise and Ladthe(5). In
the same year Blaschko proposed a hypothetical series of reactions by which noradrenaline and adrenaline might be formed from tyrosine. The hypothetical chain suggested was as follows:

\[ \text{L-TYROSINE} \rightarrow \text{DOPA} \rightarrow \text{DOPAMINE} \rightarrow \text{NORADRENALINE} \rightarrow \text{ADRENALINE} \]

It is now well established that this proposed biosynthesis schema was correct.

**Tyrosine Hydroxylase**

The conversion of L-tyrosine to L-DOPA is catalyzed by the enzyme tyrosine hydroxylase (6). This reaction has been shown in vitro to require a tetrahydropteridine cofactor, Fe\(^{++}\) and oxygen. This enzyme does not hydrolyze D-tyrosine, tyramine or tryptophan. There are two classes of compounds which inhibit this enzyme, amino acids and catechols (7). It was found that catechol inhibition of the enzyme is not competitive with the substrate but with the pteridine cofactor. The catalysis of L-tyrosine to L-DOPA is the rate limiting step in the biosynthesis of noradrenaline.

**DOPA-decarboxylase**

The decarboxylation of L-DOPA is catalyzed by the enzyme DOPA-decarboxylase (8,9). This enzyme is not specific and is widely distributed in the peripheral tissues and the brain.

**Dopamine-beta-hydroxylase**

Dopamine-beta-hydroxylase is the enzyme responsible for converting dopamine to noradrenaline (10). The localization of
this enzyme seems to be in the storage granules(11). Several other phenylethylamine derivatives can also be oxidized by this enzyme, for example, tyramine and alpha-methyl-dopamine(12). A metal ion may be important in the activity of this enzyme. The ion seems to be Cu^{++} although there is reason to suspect Fe^{++} or Co^{++}(13).

Inactivation

Our knowledge of the metabolic degradation of catecholamines has largely increased during the last decade. A schema of the metabolic inactivation pathway of noradrenaline is given in figure 1.

Monoamine Oxidase

In 1937 Blaschko, Rickter and Scholssman discovered an amine oxidase with the ability to inactivate adrenaline(14). Rickter showed that this enzyme was able to oxidize many other amines(15). The enzyme catalyzing the oxidative deamination of monoamines was later called monoamine oxidase (MAO). In 1957 Blaschko(16) suggested that there might exist metabolic pathways of monoamine degradation other than oxidative deamination. Almost at the same time acid catecholamine metabolites were detected in the urine, namely 3,4-dihydroxymandelic acid and 3,4-dihydroxyacetic acid(17). The enzyme MAO is widely distributed in the tissue and is thought to be largely localized in the mitochondria(18). The deaminated metabolite formed by this enzyme is an aldehyde(19), which can either be reduced to an ethanol or a glycol by the enzyme aldehyde dehydrogenase.
Figure 1.
Pathway for metabolism of noradrenaline (21).
The noradrenaline released from the firmly bound store is deaminated by the mitochondrial MAO in the sympathetic nerves(20). Thus noradrenaline leaves the nerve as a physiologically inactive deaminated catechol.

Catechol-O-Methyl Transferase

The 3-0-methylating enzyme, catechol-O-methyl transferase (COMT) catalyzes the transfer of methyl groups from S-adenosylmethionine to the meta-hydroxyl group of catecholamines. The enzyme is thought to be localized outside the neuron in the peripheral tissue(22).

When the purified enzyme COMT was incubated with adrenaline, S-adenosylmethionine and Mg++, one mole of metanephrine was formed for each mole of epinephrine metabolized. The enzyme had an absolute requirement for Mg++ or other divalent cations such as Mn, Co, Zn, Fe, or Ni. All catechols were O-methylated regardless of the substituent on the aromatic nucleus(23). The enzyme does not show specificity toward the d or l isomer.

The distribution of COMT is widespread. It is present in various tissues, glands, blood vessels, sympathetic and parasympathetic nerves and ganglia, and all areas of the brain. The main site of O-methylation of circulating catecholamines is in the liver(24). The compounds can also be O-methylated locally in other tissues in vivo. There are numerous studies which show that O-methylation is an important step in the metabolism or noradrenaline and adrenaline(25, 26, 27). The methods
used have consisted of injecting a physiological dose of noradrenaline or adrenaline and measuring normetanephrine and deaminated products formed in the urine or in the whole animal. Almost all of the noradrenaline and adrenaline normally formed in the body is metabolized and excreted as O-methylated deaminated products. The daily excretion of endogenous O-methylated metabolites range from 2 to 4 mg. for 3-methoxy-4-hydroxymandelic acid (VMA)(28), 100 to 300 ug. for normetanephrine and 100 to 200 ug. for metanephrine. Most of the VMA presumably arises from the deamination of noradrenaline within the sympathetic nerves followed by O-methylation, probably outside the nerves. The VMA in the urine probably represents the amount of noradrenaline produced and metabolized before it had a chance to produce a physiological effect. The normetanephrine largely represents the amount of physiologically active noradrenaline that was discharged from sympathetic nerves. Inhibition of COMT results in a prolongation of the pressor action of catecholamines(9).

Uptake of Catecholamines by Sympathetic Nerves

The possibility that exogenous catecholamines might be taken up into storage sites in the peripheral tissues was suggested by Burn(29). It was not until radioactively-labelled catecholamines of high specific activity became available that experiments could be performed using injected doses of adrenaline and noradrenaline small enough to be comparable to amounts likely to be encountered under physiological conditions. The
first demonstration of tissue uptake was made by Axelrod, Weil-Malherbe and Tomchick(30). Their results showed that after intravenous administration of H3-adrenaline a substantial proportion of the injected dose was inactivated by a rapid transfer from the circulation into peripheral tissue. In a subsequent study Whitly, Axelrod and Weil-Malherbe(31), performed similar experiments with H3-noradrenaline. They found that tissue uptake operated to remove the intravenously administered catecholamine from the circulation. The accumulation of noradrenaline in the tissue was found to be greater than that of adrenaline. The thought then prevailed that catecholamine uptake might represent an important mechanism for the physiological inactivation of catecholamines.

There was evidence that catecholamine uptake occurs in sympathetic nerves(31). Whitly found that after noradrenaline infusion, uptake of noradrenaline was greatest in the tissue with rich sympathetic innervation such as the heart. In the guinea pig heart and in the isolated perfused cat heart there is a significant correlation between the amounts of H3-noradrenaline taken up by the tissue and the endogenous content of noradrenaline(32).

In tissues in which a normal sympathetic nerve supply is lacking, the ability to take up exogenous catecholamines is severely impaired. After superior cervical ganglionectomy the uptake of catecholamines in tissues innervated by this ganglion is severely reduced(33). The uptake of H3-noradrenaline is also markedly reduced in various tissues of immunosympathectomized
rats and mice, in which the development of the sympathetic nervous system was suppressed by administration of nerve growth factor antiserum to newborn animals (34). These findings suggest that the uptake of catecholamines occurs mainly in sympathetic nerve terminals, but some caution should be used in making this interpretation. In all denervation experiments a small uptake of noradrenaline has been found in the denervated tissues.

There are radioautographic and fluorescent histochemical techniques to show that noradrenaline uptake is localized in postganglionic sympathetic nerve terminals and also in postganglionic sympathetic nerve cell bodies in the cervical sympathetic ganglion.

The Adrenergic Receptor

Ahlquist (35) in 1948 first combined results of two procedures to classify operationally different types of adrenergic receptors. The procedures used for differentiating types of adrenergic receptors are of two kinds. In the first procedure, dose-response curves of a given effector system were obtained for adrenaline, noradrenaline and closely related amines; and the relative potencies of these agonists were compared. In the second procedure, the ability of various drugs to antagonize or block the response of the effector system to one or more of the agonists was determined. Ahlquist concluded that most adrenergic receptors were either one of two types which he termed alpha or beta.
Alpha-Adrenergic Receptor

The alpha-adrenergic receptor is most responsive to adrenaline and least responsive to isoproterenol. It is blocked by the classic adrenergic blocking agents such as phenoxybenzamine and phentolamine. The common effector responses associated with this receptor are:(36)

1. Vascular smooth muscle contraction (vasoconstriction). This response can be obtained in all vascular beds but is most prominent in the skin and kidney. This response has been applied with the greatest success to demonstrate Alpha Adrenergic Activity(37).
2. Iris radial muscle contraction (mydriasis). Recent evidence indicates that only alpha-receptors are involved with this structure.
4. Orbital smooth muscle contraction. This apparent exophthalmos is best seen in the cat.
5. Splenic smooth muscle contraction. A decrease in spleen size occurs in all species but only in some species does an increase in hematocrit occur.
6. Myometrial contraction. This occurs in all species but is a prominent response in female humans, rabbits, dogs and pregnant cats.
7. Retractor penis contraction.
8. Seminal vesicle contraction.
10. Intestinal smooth muscle relaxation.
Beta-Adrenergic Receptor

This receptor is most responsive to adrenaline if only the naturally occurring catecholamines are considered, in general noradrenaline is much less potent. Isoproterenol is more potent than adrenaline on the beta-receptor. This receptor is blocked by such agents as dichloroisoproterenol and pronethalol, but is unaffected by the alpha-adrenergic blocking agents. Some of the effector responses associated with beta-adrenergic receptors are: (37)

1. Vascular smooth muscle relaxation (vasodilation). This response occurs in all vascular beds but is most prominent in skeletal muscle.
2. Myocardial positive inotropic response.
4. Myometrial relaxation. This occurs in all species but is a prominent response in female rats and non-pregnant cats.
5. Intestinal smooth muscle relaxation.

Cardiovascular Pharmacology

Many factors influence the cardiac output and vascular resistance which in turn influence blood pressure. A few factors will be described (38).

Figure 2 shows that the minute output of the ventricle is immediately determined by the stroke volume and the frequency of contraction of the ventricle. The stroke volume is determined by arterial pressure, strength of the ventricle muscle and diastolic ventricular volume. The diastolic volume
is in turn determined by venous filling pressure, diastolic filling time, compliance of the ventricular wall during diastole and resistance to blood flow through the atrio-ventricular valve. It can be stated that cardiac output is influenced by several immediate and remote mechanical factors. Some factors can be influenced by a chemical such as noradrenaline or by the sympathetic nervous system, postganglionic fibre, the mediator of which is noradrenaline.

Mechanical forces involved in filling

- FILLING PRESSURE
- FILLING TIME
- VENTRICULAR COMPLIANCE
- VALVULAR RESISTANCE

Mechanical forces which determine the minute output of the ventricles(38).
The resistance to blood flow through the entire systemic vascular bed influences arterial pressure which in turn affects cardiac output (figure 3). The resistance is immediately determined by the geometric and viscous component of resistance. The vessel radius is the most important variable in the geometric component of resistance. Radius is influenced by the contractile state of vascular smooth muscle. A chemical such as noradrenaline or isoproterenol may affect resistance by altering the contractile state of vascular smooth muscle.

Noradrenaline in the intact animal has little regular effect on the cardiac output. A fall in contraction frequency is often balanced by a rise in stroke volume. The fall in frequency in the intact animal results reflexly via the baroreceptors
mechanism and possibly via some effect on the central nervous system. Administration of noradrenaline intraventricularly into cerebrallateral ventricles produced bradycardia and hypotension(38). The increase in stroke volume may be attributed to an increase in contractile force. The increase in force may result from an increase in strength of the muscle.

Noradrenaline in the intact animal affects the peripheral circulation by raising the total peripheral resistance. This rise in peripheral resistance results predominantly from reduction in net blood vessel radius due to activation on vascular smooth muscle. The venous smooth muscle is also activated which is indicated by a decrease in venous compliance. There is an elevation of right atrial pressure which probably results from generalized decrease in venous radius, compliance or both. The net result is an increase in blood pressure.

Isoproterenol and Pronethalol

Isoproterenol is a drug with primarily beta-mimetic action. The isopropyl group attached to the N-atom seemed to suggest that this catecholamine would not undergo deamination and would be metabolized exclusively by catechol-O-methyl transferase. Sjoerdsma(39) used the D isomer of isoproterenol to measure COMT activity of hypertensive patients by giving isoproterenol and isolating the 3-methoxy-isoproterenol. Later Hertting(40) showed that $^{3}$H-isoproterenol injected in the rat, 65% of the administered activity was excreted in the urine and 35 to 45% of the administered activity was excreted in the bile.
The urine contained $^3$H-isoproterenol, $^3$H-isoproterenol glucuronide, free methoxy-$^3$H-isoproterenol and 3-methoxy-$^3$H-isoproterenol glucuronide. In the bile only 3-methoxy-$^3$H-isoproterenol glucuronide was found. Tissue studies revealed that most of the activity present in the tissue ten minutes after the injection of $^3$H-isoproterenol was already in the O-methylated form. Tissue uptake of isoproterenol was confirmed to be of insignificant amount(41). After intravenous injection of isoproterenol, small amounts were found in the heart after ten minutes, but most of this disappeared in two hours. Isoproterenol is poorly bound by intracellular particles and readily removed by post perfusion.

The beta-adrenergic blocking agents are structurally similar to the beta-adrenergic agonist isoproterenol. The side chain of pronethalol is similar to that of isoproterenol.

One of the most important features of the beta-adrenergic blocking drugs is their relatively high degree of specificity. For example although they block the positive inotropic and chronotropic effects of adrenergic stimuli, they do not block the cardiac stimulating effects of calcium, methyl xanthine or digitalis glycosides. Similarly whereas vasodilation in response to injected isoproterenol is blocked, vasodilation in response to histamine, nitroglycerine or acetylcholine is unaffected(42). The vasodilatation and cardiac acceleration effects of isoproterenol were effectively blocked by pronethalol, but there was no inhibition of the vasoconstrictor response to noradrenaline(43). These findings were
consistent with the hypothesis that pronethalol inhibited only beta-adrenergic receptors.

The half life for the block of isoproterenol tachycardia produced by pronethalol in rabbits was 36 to 45 minutes, whereas the metabolic half life was 60 to 70 minutes\(^{44}\).

In addition to pronethalol several other beta adrenergic receptor blocking agents have been developed\(^{44}\). All these compounds have been found to exert a competitive type of blockade; that is, the dose-response curve of the agonist is shifted progressively to the right with increasing concentration of the agonist. The beta-adrenergic blocking agents dichloro-isoproterenol and pronethalol prevent the uptake of noradrenaline infused into organs\(^{45}\).

**Noradrenaline and Phenoxybenzamine**

Noradrenaline causes bradycardia of reflex origin. There is a rise in both systolic and diastolic blood pressure during injection of noradrenaline, a reflection of generalized vasoconstriction. Cardiac output remains unchanged or falls\(^{22}\).

Various drugs have been shown to abolish the pressor action of noradrenaline in anaesthetized animals by antagonizing its vasoconstrictor action. This has been demonstrated for ergot alkaloids, yohimbine, tolazoline, phentolamine, phenoxybenzamine and dibenamine. It has also been demonstrated that phenoxybenzamine in doses which abolish the pressor action of noradrenaline in normal anaesthetized cats and rats fail to abolish the pressor action of noradrenaline in preparations
that have been pithed. This is due to the increase in cardiac output (46). Another action of alpha-adrenergic blocking agents such as phenoxybenzamine has been shown to prevent the uptake of infused noradrenaline (47).

The term irreversible competitive antagonism or non-equilibrium antagonism have both been used to designate the type of antagonism exerted by phenoxybenzamine against drugs acting on a number of different types of receptors. In this type of antagonism the final complex formed between receptor and antagonist does not reversibly dissociate (48), and therefore mass action equilibrium, such as is assumed to occur in classical competitive antagonism is not possible. However, two reports noted that dichlorisoproterenol could antagonize the blocking action of some alpha-adrenergic blocking agents on the pressor effect of adrenaline and noradrenaline. In 1965 another report appeared which stated that pronethalol reversed the inhibitory effect produced by phenoxybenzamine on the pressor response to adrenaline and noradrenaline (49). This effect is particularly intriguing in the case of phenoxybenzamine, since blockade by this drug is of the non-equilibrium type, with very prolonged duration of action.

The pharmacological active form of the molecule is the ethylene immonium ion which first combines with the anionic site of the alpha receptor by an ionic bond, competing with noradrenaline and then rearranges to alkylate the receptor, thereby producing an irreversible prolonged antagonism. These mechanisms, which are shown diagramatically in figure 4, were first postulated by Belleau (50).
The two combinations of haloalkylamines with the alpha-receptor. The first combination is reversible and the antagonism of noradrenaline is competitive; the second combination is irreversible. In phenoxybenzamine, \( R = \text{CH}_2\text{C}_6\text{H}_5 \) and \( R' = \text{CH(CH}_3\text{)}\text{CH}_2\text{C}_6\text{H}_5 \).

**Tropolones**

Sanders(51) wrote an excellent review of the thujaplicins which are the natural occurring isopropyl derivatives of tropolone (I) to the year 1961. Except for some information pertinent to this thesis the literature survey included information from 1961 to the present time.

**Chemistry**

The structures of various tropolones are shown in figure 5. The natural occurring tropolones which are referred to as thujaplicins are found in the western red cedar tree; alpha-thujaplicin (II), beta-hydroxy-thujaplicin (V), beta-thujaplicin (III), and gamma-thujaplicin (IV). The investigation of beta-hydroxy-thujaplicin (7-hydroxy-4-isopropyltropolone) (V) and gamma-thujaplicin (5-isopropyltropolone) (IV) is described in this thesis.
A chemical property which the tropolone and catechol ring have in common is the ability to form stable chelates with divalent ions (53). It has also been shown that the two rings are isosteric (52).

The thujaplicins are weak acids with titration curves typical of monobasic acids. The acidity increases from alpha to beta to gamma-thujaplicin, with $K_a$ values of 7.8, 7.3 and 7.1 respectively. In solution, beta-thujaplicin chelation will be accompanied by a drop in $pH$. The stability of chelation of beta-thujaplicin decreases in the order copper, iron (II), nickel, zinc and cobalt (II). Magnesium and zinc do not form detachable chelates with beta-thujaplicin $10^{-4}$ molar (54).
Beta-thujaplicin is moderately stable at $10^{-3}$ molar solution in water at room temperature (54). After two days about 15% decomposed and at fourteen days about 40% of the beta-thujaplicin decomposed.

Pharmacology

The pharmacology of beta-thujaplicin was investigated first by Lee in 1951 (51). In his first paper, he reported on the toxicity and local action of beta-thujaplicin and its salt. In his second paper Lee found that beta-thujaplicin caused a fall in blood pressure in guinea pigs and in rabbits. He also noticed a decrease in pulse rate and respiratory depression.

The pharmacological properties of gamma-thujaplicin were first investigated by Halliday (55). These studies consisted of toxicity determinations in mice. In addition, the effect of gamma-thujaplicin was studied on the central and peripheral nervous system, blood pressure and heart rate of the cat and dog. He found that gamma-thujaplicin 25 to 50mg/kg produced a temporary fall in blood pressure and a decrease in heart rate; while 50mg/kg produced complete paralysis of respiration. The hypotensive effect remained unchanged after atropinization or bilateral section of the vagus. Responses to carotid occlusion and to injected adrenaline were not altered by an immediately previous injection of gamma-thujaplicin.

Belleau (52) was the first to show in vitro that tropolones were inhibitors of COMT. He had also shown that the reaction was of a competitive nature, later confirmed by
D'Jario(56). The inhibitory mechanism of COMT is thought to involve the enzyme bound magnesium and the tropolone. Belleau thought that a 1:1:1 complex is formed between COMT, magnesium and inhibitor. 4-methyltropolone was found to be the most active COMT inhibitor whereas beta-thujaplicin was of moderate activity. It was later found that beta-thujaplicin does not form chelates with magnesium(55). Gamma-thujaplicin and beta-hydroxy thujaplicin showed moderate COMT inhibition. Ross(57) found that 4-methyltropolone (VI) 10mg/kg. and beta-thujaplicin 20mg/kg. could cause COMT inhibition in mice. Doses as high as 100mg/kg. produced no adrenergic blockade in these animals.

Studies in mice indicated that 4-methyltropolone in small doses increased the percent mortality of mice injected with adrenaline; this may be due to inhibition of COMT. Large doses exert a protective effect which might be due to a transitory blockade of the alpha-receptors. In the same study it was also found that methyltropolone reduced the vasodepressor effect of isoproterenol(58). Murnaghan in 1964(59) showed that 4-methyltropolone and beta-thujaplicin in small doses increased the height of contraction of the aortic strip of the rabbit when stimulated with adrenaline and potentiated the toxic effect of adrenaline in mice; in large doses they antagonize the adrenaline induced death in mice. Methyltropolone also potentiates and antagonizes respectively potassium and barium induced contractions on the aortic strip. They therefore concluded that the mechanism of the adrenaline potentiation cannot be due to inhibition of COMT. They felt that antagonism and
potentiation of adrenaline were due to non specific effects of 4-methyltropolone and beta-thujaplicin.

Gamma-thujaplicin as mentioned previously is a chelating agent which complexes with various divalent ions, but is most stable with copper. The tropolones have been shown to be potent inhibitors of dopamine hydroxylase in vitro\(^{(13)}\). Since this enzyme is located in the storage site of noradrenaline, it would seem that these tropolones could enter this part of the neurone. Wyse and Halliday\(^{(60)}\) reported that gamma-thujaplicin in vivo lowered the copper content of rat heart tissue. They had shown that the responses of the rat atria to tyramine had been reduced when treated with gamma-thujaplicin. They then concluded that their data was consistent with the hypothesis that chelating agents lower tissue catecholamines due to chelation of heavy metals necessary for the activity of enzymes involved in catecholamine synthesis.
EXPERIMENTAL

Method

Male Wistar rats weighing 300 to 500 grams were used for these experiments. The operative technique used was similar to that originally described by Landgrebe(61) and modified by Dekanski(62). Initially pentobaritone sodium 50mg/kg. was employed as an anesthetic, but anesthesia levels varied greatly from animal to animal and sometimes a supplemental dose was required. Urethane 1.3 to 1.5gm/kg. was found to be a more satisfactory anesthetic and was used for the majority of experiments. The rat was chosen for these experiments because the limited quantities of gamma-thujaplicin and beta-hydroxy thujaplicin available did not permit the use of a larger experimental animal.

Operative Procedure

The fore and hind legs were secured to a warmed table and rectal temperature was maintained from 33 to 35 degrees centigrade. The left carotid artery and the trachea were dissected, ready for cannulation. The trachea was cannulated with a length of polyethylene tubing of size PE 240. A femoral vein was cannulated with polyethylene tubing of size PE 60, which was connected by a hypodermic needle to a three way stopcock. One of the remaining arms of the stopcock was connected to a 25 ml. burette, the other to a syringe used for the injection.
of chemicals. Heparin 1.5mg/100gm. of body weight was injected intravenously via the femoral vein cannula. The left carotid artery was then cannulated with a polyethylene tube of size PE 90, which was connected through a saline-filled tube to a Condon mercury manometer, or to a Statham model P 23 series pressure transducer. A model 5 Grass Polygraph, sensitivity 10, or a Kymograph was used for recording blood pressure.

After the operative procedure, one hour was allowed for the blood pressure and heart rate to stabilize. Pentolinium tartrate, a ganglionic blocking drug, was administered in the dose of 0.125mg/rat to prevent fluctuation of blood pressure(63). Pentolinium tartrate was not administered to rats in experiments conducted to find the effect of gamma-thujaplicin or beta-hydroxy thujaplicin on the vasopressor response to physostigmine.

In pithed animals, pithing was done under ether according to the method of Shipley and Tilden(64), prior to exposure and cannulation of blood vessels. Pithed animals were maintained on a Harvard Series 680 Rodent Respiratory pump. Respiration was maintained at 60 cycles per minute, the volume of air per stroke being one ml. per 100 grams of body weight.

Heart rate was monitored by a Sanborn Viso Cardlette, model 51 with readings taken every minute. Later a model 5 Grass Polygraph was used with a model 5P6 EKG preamplifier.

Drugs Employed

The following drugs were employed; noradrenaline bitartrate, isoproterenol hydrochloride, gamma-thujaplicin sodium,
beta-hydroxy thujaplicin sodium, phenoxybenzamine hydrochloride, pronethalol hydrochloride, pentolinium tartrate, heparin sodium and physostigmine salicylate. The doses used are expressed in terms of salts employed. All solutions were made with normal saline prior to each experiment.

The sodium salt of beta-hydroxy thujaplicin was prepared according to the method of Halliday (55).

Tabulation of Heart Rate and Blood Pressure Data

Measurements tabulated are the maximum changes, either increases or decreases of blood pressure (mm.Hg.) or heart rate (beats per minute), from the baseline. The baseline and baserate respectively is taken as the blood pressure or heart rate immediately before an injection of a drug. The duration of response indicates the time in minutes from the initial rise until the blood pressure had returned to within 5 mm.Hg. of the baseline and the heart rate to within 20 beats per minute of the baserate.

In all statistical data the "t" test was used with a level of significance of 0.10 and the standard error of the mean tabulated. The + or - refers to an increase or a decrease of blood pressure or heart rate from the baseline. Control injections of normal saline 0.5 ml. were made in each animal. Values for the effects of these control injections are given only for animals in which a response occurred.
Evaluation of Alpha-Receptor or Beta-Receptor Blockade

In experiments where pronethalol 10mg/kg. was used, isoproterenol was used in the following manner to test the effectiveness of beta-receptor blockade. A test response to 0.25ug/kg. of isoproterenol was obtained before administration of pronethalol. Following pronethalol, the absence of a response, or presence of only a slight response to 0.5ug/kg. of isoproterenol was considered to indicate the presence of an adequate beta-receptor blockade. The same procedure using noradrenaline as the test drug was used for evaluating the effectiveness of alpha-receptor blockade by phenoxybenzamine 5mg/kg.

Results

Gamma-Thujaplicin

Effect on Blood Pressure and Heart Rate

A. Anesthetized Rats

Gamma-thujaplicin 30mg/kg. produced either a vasodepressor or a vasopressor response. Tachycardia occurred in all experiments following gamma-thujaplicin administration.

A vasopressor response was produced in six rats out of twelve animals (fig. 6). The average increase in blood pressure was 29.5 mm. Hg. or 55% of control pressure, which occurred one-half minute after an injection of gamma-thujaplicin. The average duration of this response was two minutes. The magnitude
of the pressor response varied between animals, ranging from 6 to 60 mm.Hg. In conjunction with the pressor response tachycardia occurred. The average increase in heart rate was 60 beats per minute after an injection of gamma-thujaplicin. The peak maximum increase in heart rate occurred one-half minute after the peak change in blood pressure was reached. The duration of the tachycardia was three minutes.

![Graph showing blood pressure and heart rate changes over time](image)

**Figure 6.**

The vasopressor and tachycardiac response induced by gamma-thujaplicin 30mg/kg. in anesthetized intact rats.
In the remaining six rats gamma-thujaplicin produced a vasodepressor response (fig. 7). The average decrease in blood pressure was 12 mm.Hg. or 16% and occurred one-half minute after an injection of gamma-thujaplicin. The duration of this response was difficult to determine because the blood pressure did not return to within 5 mm.Hg. of the initial blood pressure which was 61.5±5 mm.Hg. The magnitude of the depressor response varied from minus 5 to minus 28 mm.Hg. Vasodepression was accompanied by tachycardia. The average increase in heart rate was 45 beats per minute. This response occurred one to two minutes after gamma-thujaplicin was injected. This increase in heart rate followed the vasodepressor response by one-half minute. The duration of the tachycardia was four minutes.

**Figure 7.**
The vasodepressor and tachycardiac response produced by gamma-thujaplicin 30mg/kg. in anesthetized intact rats.
Gamma-thujaplicin apparently produced complex and variable cardiovascular changes in these rats. Although the initial blood pressures and heart rates were similar, either a decrease or an increase in blood pressure occurred. In rats in which gamma-thujaplicin increased the blood pressure, the average initial blood pressure was 62.6 mm.Hg, and the average initial heart rate was 290 beats per minute. In rats in which the blood pressure was decreased, the average initial values for blood pressure and heart rate were 61.5 mm.Hg. and 316 beats per minute. Whether there was an increase or a decrease in blood pressure, the maximum increase in heart rate was of the same magnitude. It would seem that gamma-thujaplicin could influence the heart rate independently of the change in blood pressure. The tachycardiac response was more prolonged when vasodepression occurred. It was also observed that the vasodepressor response was of a greater duration than the vaso­pressor response, although both were short-lasting. Since a ganglionic blocking drug was employed it seemed unlikely that the responses could be mediated via the preganglionic autonomic neurons. Doses of gamma-thujaplicin lower than 30mg/kg did not produce a response consistently.

B. Repeated Doses

To determine whether changes in blood pressure and heart rate could be replicated in the same animal, four injections of gamma-thujaplicin 30mg/kg were administered fifteen minutes apart.
Table I

Effect on Blood Pressure and Heart Rate of Repeated Injections of Gamma-Thujaplicin 30mg/kg.

<table>
<thead>
<tr>
<th></th>
<th>Blood Pressure Increase (mm.Hg.)</th>
<th>Heart Rate Increase (beats per minute)</th>
</tr>
</thead>
<tbody>
<tr>
<td>First Dose</td>
<td>23±3.2</td>
<td>80±20</td>
</tr>
<tr>
<td>Second Dose</td>
<td>27.6±6.3</td>
<td>80±30</td>
</tr>
<tr>
<td>Third Dose</td>
<td>13.3±6.7</td>
<td>6.6±6.7</td>
</tr>
<tr>
<td>Fourth Dose</td>
<td>6.6±6.6</td>
<td>26.6±23.6</td>
</tr>
</tbody>
</table>

n=4

In all four animals an increase in blood pressure and heart rate were observed. The maximum increase in blood pressure and heart rate were similar after the first two doses of gamma-thujaplicin (table I) but were greatly reduced after the third and fourth dose of gamma-thujaplicin. A degree of tachyphylaxis appeared to have developed following the second dose of gamma-thujaplicin.

C. Pithed Rat

Four experiments were done in rats in which the brain and spinal cord were destroyed by pithing according to the method of Shipley and Tilden(65). The average initial blood pressure of the pithed rats was 34.5±1.6 mm.Hg. which was considerably lower than that of the anesthetized rats. The average initial heart rate of the pithed rats was 290±17.3 beats per minute which was similar to that in the anesthetized rats.
In all four pithed rats gamma-thujaplicin produced a fall in blood pressure and decrease in heart rate. The average decreases in blood pressure and heart rate were respectively minus 16±2 mm.Hg. and minus 46±6 beats per minute. Since all central influences on the cardiovascular system are absent in the pithed rat, the effects of gamma-thujaplicin could not have been of central origin and must be due to action at peripheral sites.

Effect of Adrenergic Blocking Drugs on the Response to Gamma-Thujaplicin

Gamma-thujaplicin 30mg/kg. was administered to obtain a control response. This was followed by administration of the appropriate blocking agent. The blocking agents used were pronethalol 10mg/kg. and phenoxybenzamine 5mg/kg. The procedure described earlier (see Experimental p.26) was employed to establish the effectiveness of receptor blockade and gamma-thujaplicin 30mg/kg. was again administered. Both intact and pithed rats were used. Since tachyphylaxis developed after two doses of gamma-thujaplicin the effect of only one blocking agent was tested in a single animal.
### Table II

The Effect of Pronethalol 10mg/kg. and Phenoxybenzamine 5mg/kg. on the Response to Gamma-Thujaplicin 30mg/kg.

<table>
<thead>
<tr>
<th>Gamma-Thujaplicin</th>
<th>Intact Rats</th>
<th>Pithed Rats</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Maximum Change in</td>
<td>Maximum Change in</td>
</tr>
<tr>
<td></td>
<td>B.P.</td>
<td>H.R.</td>
</tr>
<tr>
<td></td>
<td>mm.Hg.±S.E.</td>
<td></td>
</tr>
<tr>
<td>Control Response</td>
<td>+15±6.6</td>
<td>+36±21.6</td>
</tr>
<tr>
<td>After</td>
<td>+3.3±3.5*</td>
<td>+8±13.5</td>
</tr>
<tr>
<td>Phenoxybenzamine</td>
<td>n=5</td>
<td>n=5</td>
</tr>
<tr>
<td>Control Response</td>
<td>+10±5.3</td>
<td>+60±5.1</td>
</tr>
<tr>
<td>After</td>
<td>-5±9.5*</td>
<td>+15±9.4</td>
</tr>
<tr>
<td>Pronethalol</td>
<td>n=4</td>
<td>n=4</td>
</tr>
</tbody>
</table>

*"t" test (paired), p=0.10, significant

B.P. = blood pressure
H.R. = heart rate, beats per minute
n = number of animals

It can be seen from table II that the vasopressor effect of gamma-thujaplicin in anesthetized rats was significantly reduced by phenoxybenzamine 5mg/kg. The control vasopressor response to gamma-thujaplicin was +15.4 mm.Hg. which was reduced to +3.3 mm.Hg. by phenoxybenzamine. The control increase in heart rate produced by gamma-thujaplicin was +36 beats per minute. This response was reduced to +8 beats per minute in the presence of phenoxybenzamine but, this change was not statistically significant. The initial blood pressure which was 58.5 mm.Hg. before phenoxybenzamine was administered was
reduced to 40 mm.Hg. after phenoxybenzamine 5mg/kg. The initial heart rate was increased from 320 beats per minute to 370 beats per minute by phenoxybenzamine.

In anesthetized rats pronethalol 10mg/kg. reversed the vasopressor effect produced by gamma-thujaplicin 30mg/kg. to one of vasodepression. The increase in blood pressure of 10 mm.Hg. produced by gamma-thujaplicin was reduced to a decrease of 5 mm.Hg. in the presence of pronethalol, a change which was statistically significant. Although pronethalol reduced the increase in heart rate produced by gamma-thujaplicin from 60 beats per minute to 15 beats per minute the reduction was not statistically significant. Pronethalol itself caused little change in blood pressure. The blood pressure before pronethalol was 54 mm.Hg. whereas after administration of pronethalol the blood pressure was 55 mm.Hg. Pronethalol did decrease the heart rate slightly, from 375 to 300 beats per minute.

In the pithed rat pronethalol 10mg/kg. did not alter the vasodepressor response produced by gamma-thujaplicin 30mg/kg. The decrease in blood pressure produced by gamma-thujaplicin before pronethalol was 13.6 mm.Hg., whereas after pronethalol the decrease was 14.5 mm.Hg. It can then be assumed that the decrease in blood pressure produced by gamma-thujaplicin in the pithed rats was not due to beta-receptor stimulation. In the pithed rat gamma-thujaplicin produced a significantly greater decrease in heart rate after treatment with pronethalol. The control decrease in heart rate produced
by gamma-thujaplicin was 47 beats per minute whereas the decrease in heart rate after treatment with pronethalol was 70 beats per minute. This greater decrease in heart rate produced after pronethalol administration might be explained as being due to additive effects of the two drugs since pronethalol has been shown to produce bradycardia by direct myocardial depression(65) and beta-adrenergic blockade.

Effect of Gamma-Thujaplicin on Responses to Noradrenaline and Isoproterenol

The following procedure was used on anesthetized rats. Two doses of noradrenaline 0.5ug/kg. were given twenty minutes apart, followed by two injections of isoproterenol 0.25ug/kg. twenty minutes apart. Gamma-thujaplicin 7mg/kg. was then administered. Ten and thirty minutes after the administration of gamma-thujaplicin, noradrenaline 0.5ug/kg. and isoproterenol 0.25ug/kg. respectively, were again given.

The results obtained are recorded in figure 8 and 9 as maximum changes in blood pressure. Two control injections of noradrenaline (fig. 8) and isoproterenol (fig. 9) were given per animal. The average of their responses was calculated.

The control vasopressor response to noradrenaline was 32±6.8 mm.Hg. The vasopressor responses to noradrenaline ten and thirty minutes after gamma-thujaplicin were 18±5.01 mm.Hg. and 14.2±5.9 mm.Hg. respectively, both of which are significantly lower than the control responses.
The vasodepressor response to isoproterenol before gamma-thujaplicin was minus 21.5±1.5 mm.Hg. Responses of minus 21.4±3.2 mm.Hg. and minus 24.5±2 mm.Hg. were obtained ten and thirty minutes after the administration of gamma-thujaplicin.
It would appear from these results that gamma-thujaplicin can inhibit the action of noradrenaline on the rat blood pressure but has little or no effect on the action of isoproterenol.
Effect of Gamma-Thujaplicin on the Vasopressor Response to Physostigmine

The pressor response to intravenously injected physostigmine in the rat arises from activation of the central sympathetic mechanism (66, 67, 68) and in many ways is similar to the effect of sympathetic nerve stimulation. The pressor response is blocked by bretylium, guanethidine and syrosingopine(69). Physostigmine salicylate 40ug/kg. when injected intravenously produced a pressor effect which was constant when repeated in the same rat but was variable between rats.

Two control responses to physostigmine 40ug/kg. were obtained thirty to forty minutes apart. A third injection of physostigmine was given twenty minutes following administration of gamma-thujaplicin. Pentolinium tartrate was not administered in these rats since blockade of sympathetic ganglia would inhibit the pressor response to physostigmine.

Table III
Effect of Gamma-Thujaplicin on the Vasopressor Response to Physostigmine.

Maximum Change in Blood Pressure (mm.Hg.±s.e.)

<table>
<thead>
<tr>
<th></th>
<th>Before Gamma-Thujaplicin</th>
<th>After Gamma-Thujaplicin</th>
</tr>
</thead>
<tbody>
<tr>
<td>Physostigmine</td>
<td>+28.5±4.3</td>
<td>+31±8.6</td>
</tr>
</tbody>
</table>

n=2
The physostigmine response before gamma-thujaplicin was very similar to the response obtained after gamma-thujaplicin (table III). Since gamma-thujaplicin did not change the response to physostigmine it can be assumed that it does not antagonize the central stimulant effect of physostigmine or affect peripheral sympathetic transmission of impulses to the cardiovascular system.

Beta-Hydroxy Thujaplicin

Effect on Blood Pressure and Heart Rate

A. Anesthetized Rats

Beta-hydroxy thujaplicin 10mg/kg. caused a vasopressor response in all nine of the anesthetized intact rats in which it was tested. Figure 10 illustrates this response. The average increase in blood pressure was 16 mm.Hg. and occurred three minutes after an injection of beta-hydroxy thujaplicin. The duration of the pressor response was seven minutes. There was no change in heart rate in any of the animals. The initial blood pressure was 68±4.1 mm.Hg.; the heart rate was 340±18 beats per minute.

B. Pithed Rats

Beta-hydroxy thujaplicin 10mg/kg. produced a pressor response in pithed rats also (table IV). There was no change in heart rate. The initial blood pressure was 34±6.2 mm.Hg. which was less than the initial blood pressure of the intact rats. The initial heart rate was 273±10.6 beats per minute.
which also was less than the initial heart rate of the intact rats. Since in the pithed rat central responses which control the cardiovascular system were abolished, the response to beta-hydroxy thujaplicin in these animals must be of peripheral origin.

![Graph](image)

**Figure 10.**

The vasopressor effect of beta-hydroxy thujaplicin 10mg/kg. in anesthetized intact rats (mm.Hg.±s.e.)

n=9

C. Repeated Doses

To determine whether replication of changes in blood pressure could be obtained in each animal, injections of beta-hydroxy thujaplicin 10mg/kg. were administered every thirty minutes to three intact and three pithed rats. The results obtained appear in table IV.
Table IV

Effect on Blood Pressure of Repeated Injections of Beta-Hydroxy Thujaplicin 10mg/kg.

<table>
<thead>
<tr>
<th></th>
<th>Maximum Change in Blood Pressure (mm.Hg.±s.e.)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>First Dose</td>
</tr>
<tr>
<td>Intact Rat n=3</td>
<td>+8±0</td>
</tr>
<tr>
<td>Pithed Rat n=3</td>
<td>+7±0.58</td>
</tr>
</tbody>
</table>

It can be seen that values in Table IV are similar for intact and pithed rats upon repeated administration of beta-hydroxy thujaplicin. Tachyphylaxis did not appear to develop in either intact or pithed rats when three successive doses were given.

Effect of Phenoxybenzamine on the Response to Beta-Hydroxy Thujaplicin.

Beta-hydroxy thujaplicin 10mg/kg. was administered to five anesthetized rats, followed by phenoxybenzamine 5mg/kg. After the effectiveness of the alpha-receptor blockade was established, in the manner previously described, beta-hydroxy thujaplicin 10mg/kg. was again administered. The results appear in Table V.

Administration of phenoxybenzamine caused a marked reduction in the effect of beta-hydroxy thujaplicin on blood pressure. Since the response to beta-hydroxy thujaplicin following the blocking agent was no different than that caused by
saline, complete inhibition of the effect of beta-hydroxy thujaplicin appears to have occurred.

Table V

Effect of Phenoxybenzamine 5mg/kg. on the Vasopressor Response to Beta-Hydroxy Thujaplicin 10mg/kg.

<table>
<thead>
<tr>
<th></th>
<th>Before Phenoxybenzamine</th>
<th>After Phenoxybenzamine</th>
</tr>
</thead>
<tbody>
<tr>
<td>Beta-Hydroxy Thujaplicin</td>
<td>+24±7.7</td>
<td>+6±2.6*</td>
</tr>
<tr>
<td>Normal Saline</td>
<td>+5.5±1.3</td>
<td></td>
</tr>
</tbody>
</table>

* "t" test (paired), n=5, p=0.05, significant

Effect of Beta-Hydroxy Thujaplicin on Isoproterenol Induced Tachycardia

The experiment consisted of two injections of isoproterenol 0.25ug/kg, twenty minutes apart. Beta-hydroxy thujaplicin 10mg/kg, was then given followed at ten and thirty minutes by isoproterenol 0.25ug/kg. Heart rate was monitored. The average increase in heart rate and duration of the tachycardiac response are shown in Table VI.

Ross(57) demonstrated that COMT inhibition by a drug could be shown by the following technique. Using an electrocardiogram to record heart rate of anesthetized mice, Ross found that the tachycardiac effect of intravenous isoproterenol was prolonged by a previous injection of 4-methyltropolone. He also found that tissue COMT activity was reduced by treatment
with tropolones and concluded that the prolongation of isoproterenol action was due to inhibition of this enzyme. However, as can be seen from Table VI, beta-hydroxy thujaplicin did not appear to influence the tachycardiac response to isoproterenol in these experiments in anesthetized rats.

Table VI

Effect of Isoproterenol 0.25μg/kg. Before and After Treatment with Beta-Hydroxy Thujaplicin 10mg/kg.

<table>
<thead>
<tr>
<th>Isoproterenol Induced Tachycardia</th>
</tr>
</thead>
<tbody>
<tr>
<td>Before Beta-Hydroxy Thujaplicin</td>
</tr>
<tr>
<td>Heart Rate</td>
</tr>
<tr>
<td>beats/minute</td>
</tr>
<tr>
<td>+85±5.7</td>
</tr>
<tr>
<td>After Beta-Hydroxy Thujaplicin</td>
</tr>
<tr>
<td>Heart Rate</td>
</tr>
<tr>
<td>beats/minute</td>
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<td>+90±16.3</td>
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Effect of Beta-Hydroxy Thujaplicin on the Vasopressor Response to Physostigmine

Using the same procedure as described under gamma-thujaplicin it was found that administration of physostigmine 40μg/kg. increased the blood pressure by 47±13.7 mm.Hg. before beta-hydroxy thujaplicin and 46.5±12 mm.Hg. after administration of beta-hydroxy thujaplicin. Four animals were used. It can be concluded that beta-hydroxy thujaplicin had no effect on the central stimulant action of physostigmine.
DISCUSSION

A survey of the literature suggests that various tro-
polones may produce, at different dose levels, a diverse number
of effects in animals. Some of these effects appear to involve
adrenergic mechanisms such as COMT inhibition (57, 52), inhi-
bition of dopamine hydroxylase(13), transitory blockade of
alpha-receptors(58) and reduction of the vasodepressor effect
produced by isoproterenol(58). In the study which has been
described here, it was found that gamma-thujaplicin and beta-
hydroxy thujaplicin had certain actions on the cardiovascular
system of the rat. In the case of gamma-thujaplicin the ef-
fects were not consistent in all animals.

Gamma-Thujaplicin

In experiments using anesthetized rats gamma-thujaplicin
30mg/kg. produced an increase in blood pressure in some animals
and a decrease in others. The type of response did not appear
to depend on the level of the initial blood pressure, since
this was similar in all rats. The heart rate was increased to
the same degree in all animals regardless of whether the blood
pressure was increased or decreased. Gamma-thujaplicin must
have some influence on the heart either through the central
nervous system or through peripheral mechanisms which is inde-
pendent of changes in blood pressure.
Halliday(55) reported that gamma-thujaplicin produced central nervous stimulant activity in the form of convulsions which were abolished when the rat was decerebrated. This is evidence that gamma-thujaplicin can pass through the blood brain barrier and into the central nervous system. The pithed rat was used to investigate the possible role of the central nervous system in the actions of gamma-thujaplicin in intact animals.

In the pithed rats, gamma-thujaplicin slowed the heart and lowered the blood pressure. In these rats, in which the brain and spinal cord are destroyed, it must be assumed that these effects are due to peripheral actions on the heart and blood vessels. The magnitude of the decrease in blood pressure in the pithed rat was of the same order as that in the anesthetized intact rat. It would appear that the vasodepression in the intact rat could be due to peripheral action on the blood vessels. It also must be assumed that the increase in heart rate and vasopressor actions produced by gamma-thujaplicin in the anesthetized rats were of central origin since they do not occur after destruction of the brain and spinal cord. It would seem then that gamma-thujaplicin can exert both central and peripheral actions which oppose one another on the cardiovascular system. In the intact anesthetized rats, where both of these mechanisms would be operational, the effects produced must represent the balance of the opposing actions. This would explain the fact that in some anesthetized rats the blood pressure was depressed, while in others it was increased. It
could be assumed that in the former the peripheral action on the vessels overcame the central action. The occurrence of central effects in the presence of a ganglionic blocking agent would indicate that ganglionic blockade was not complete. It is possible that varying degrees of sympathetic ganglionic blockade may be partly responsible for the different blood pressure responses.

Inhibition of the vasopressor action of gamma-thujaplicin by phenoxybenzamine suggests that this action is mediated through the postganglionic-sympathetic fibre to the blood vessels since phenoxybenzamine is an alpha-adrenergic blocking agent. The reduction of the vasopressor response to gamma-thujaplicin by pronethalol was unexpected and cannot readily be explained. Interference with the action of gamma-thujaplicin at whatever receptors it is acting on centrally may be a possibility.

The reduction in blood pressure produced by gamma-thujaplicin in pithed rats apparently did not involve an action on beta-adrenergic receptors since it was not antagonized by pronethalol. The intensification by pronethalol of the bradycardia produced by gamma-thujaplicin in these rats is probably due to additive effects of the two drugs, since pronethalol itself, by virtue of beta-adrenergic blocking activity and direct myocardial depressant effects can cause bradycardia. Gamma-thujaplicin could cause direct myocardial depression since 30mg/kg. does not affect the vasodilatory response to isoproterenol and thus does not appear to have beta-receptor adrenergic blocking activity.
The results of the experiments in which gamma-thujaplicin was tested for adrenergic blocking action suggests that the tropolone derivative can block receptors acted on by circulating noradrenaline but not those occupied by noradrenaline released from sympathetic nerves. This is illustrated by the reduction, by gamma-thujaplicin, of the vasopressor response to injected noradrenaline (fig. 8) and the absence of effect by gamma-thujaplicin on the response to physostigmine which produces a vasopressor effect mediated through sympathetic nerves. The inability of gamma-thujaplicin to affect the physostigmine response is likely due to its inability at lower doses to gain access to the receptors at the neuro-effector junction of the vascular smooth muscle.

Tachyphylaxis, to the vasopressor effect, resulted from repeated administration of gamma-thujaplicin. This action is produced centrally and appears to be mediated through sympathetic nerves. Upon repeated administration of gamma-thujaplicin the drug could eventually gain access to and block the receptors at the neuro-effector junctions on vascular smooth muscle. This effect could result in partial blockade of the adrenergic alpha-receptor and tachyphylaxis to gamma-thujaplicin would occur. In single doses, gamma-thujaplicin blocked only responses to exogenous noradrenaline; however, the development of tachyphylaxis suggests that endogenous noradrenaline may be blocked by repeated administration.
Beta-Hydroxy Thujaplicin

Beta-hydroxy thujaplicin 10mg/kg. caused a mean increase in blood pressure of 16 mm.Hg. in anesthetized intact rats. The duration of the vasopressor response was longer lasting than the vasopressor response caused by gamma-thujaplicin. Unlike gamma-thujaplicin, beta-hydroxy thujaplicin did not cause any change in heart rate or a decrease in blood pressure in any of the animals used.

Beta-hydroxy thujaplicin elevated the blood pressure in both intact and pithed rats but had no effect on heart rate in either preparation. It is concluded therefore that beta-hydroxy thujaplicin acts directly on the vascular smooth muscle. Since this action was opposed by phenoxybenzamine it appears to be mediated through the alpha-adrenergic receptors. This is in agreement with results obtained in this laboratory from experiments with beta-hydroxy thujaplicin on isolated rabbit aortic strips.

The chronotropic effect of isoproterenol was the same before and after an injection of beta-hydroxy thujaplicin. It would appear that beta-hydroxy thujaplicin in the dose used did not exert beta-receptor blockade or COMT inhibition.
SUMMARY AND CONCLUSIONS

1. Intravenous administration of gamma-thujaplicin sodium 30mg/kg. to anesthetized rats caused a moderate increase in blood pressure in one-half of the animals used and a smaller decrease in blood pressure in the remaining rats.

2. The vasopressor action in anesthetized rats was of central origin and was mediated via sympathetic nerves to the alpha-adrenergic receptors in vascular smooth muscle.

3. The vasodepressor action was the result of a direct action of gamma-thujaplicin on vascular smooth muscle not involving beta-adrenergic receptors.

4. In all of the anesthetized rats this dose of gamma-thujaplicin produced an increase in heart rate which was believed due to central stimulation.

5. In pithed rats gamma-thujaplicin 30mg/kg. caused a reduction in heart rate which suggests that the compound has a direct action of myocardial depression.

6. In pithed rats the only blood pressure response observed was that of vasodepression.

7. Tachyphylaxis to the vasopressor action of gamma-thujaplicin developed in the anesthetized rat after two doses were administered.

8. Gamma-thujaplicin 7mg/kg. can produce alpha-receptor blockade to exogenous noradrenaline but blockade could not be demonstrated to endogenous noradrenaline.
9. Beta-hydroxy thujaplicin 10mg/kg. in anesthetized and pithed rats increased blood pressure by acting on the alpha-adrenergic receptors of vascular smooth muscle, but did not affect heart rate.

10. The results did not indicate that beta-hydroxy thujaplicin has either a stimulant or blocking action on beta-adrenergic receptors.

11. No evidence was obtained to indicate that beta-hydroxy thujaplicin inhibits COMT in the preparations used.
BIBLIOGRAPHY


31. Whitby et al., loc. cit.


44. Ibid.


47. Ferry, loc. cit.


