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Studies on the Cardiovascular Effects of Ambrein Pretreatment in Rats

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Abstract – The pharmacological actions of ambrein were investigated alone or in combination as a pretreatment with agonists (adrenaline, noradrenaline, acetylcholine, histamine, nicotine), antagonists (atropine, atenolol) and calcium channel blocker (verapamil) *in vivo* in anaesthetized SWR rats using blood pressure, heart rate and myocardial contractility as parameters. Ambrein in the dose range of 50-200 mg/kg to the normotensive anaesthetized rats demonstrated negative chronotropic effect and increased the myocardial contractility significantly. At the mid dose (100 mg/kg) this increase in contractile force was 36% and 44% above the normal at 30 min and 60 min intervals post-treatment, respectively. Both of the lower and high doses (50 mg/kg and 200 mg/kg) had similar effects. Furthermore, this contractile response was dose related. Also, this compound produced a considerable increase in myocardial contractility when used as a pretreatment with some agonists and antagonists. The results on blood pressure did not show a considerable change when ambrein was used alone. However, ambrein pretreatment at the dose of 100 mg/kg did not block the effects of adrenaline, noradrenaline, isoprenaline and acetylcholine on heart rate and blood pressure. On the other hand, this pretreatment attenuated the sympathoadrenal effects of nicotine significantly. Chronotropic and blood pressure changes produced by histamine were also inhibited by ambrein pretreatment. This pretreatment significantly reversed the effects of atenolol but failed to demonstrate any change in the negative chronotropic, inotropic and hypotensive responses induced by verapamil. It is concluded that ambrein induced nonselective dose dependent antagonism of the effects of some agonists and antagonists require contribution of some neuromediators. However, the positive inotropic effects of ambrein possibly involve the enhancement of slow Ca channels and/or activation of ß-adrenergic receptors in the heart. At this moment it is difficult to explain the exact mode of action of ambrein and the studies dealing with Ca channel blocker and adrenergic blocker followed by ambrein may help to define the factors which contribute to its positive inotropic effects.

Key words – Ambrein, myocardial contractility, chronotropy, agonist, antagonist.

Introduction

Ambrein, a tricyclic triterpene (ambra-13, 18 diene-8-ol, $C_{30}H_{52}O$, Mol. wt. 428) is the major constituent of ambergris, the concretion of the intestinal tract of sperm whale (*Physter catodon*) (Dannanfeldt, 1982). Ambergris had been extensively used in eastern folklore medicine. In Asia besides being used as a drug, it was also employed until recently as a spice for food and wines (Benton, 1963). Ambergris remained in principal pharmacopoeias until the end of 19th century and enjoyed great popularity in Europe too. Compounds and pastes containing this substance are thought to be an excellent curative for some nervous disorders, as well as being replenisher, restorative and aphrodisiac (Al-Gozech, 1981; Epstein, 1975; Merck Index, 1968; Ohlof, 1982; Lawrence, 1983). In folklore medicine it has been used as tonic for strengthening brain senses and as replenisher of heart (Said, 1969). Some of the reports on cholinergic/ adrenergic effects of ambergris and its fractions have been published from our laboratory (Taha, 1989). A few reports on the pharmacological effects of ambrein regarding oedema (Taha and Ginawi, 1993), nociception and mode of action (Taha, 1992; 1994), blood glucose levels (Taha, 1991), changes in plasma biochemistry (Taha *et al.*, 1995) and hormonal levels (Taha and Islam, 1994) have been published.

Recently ambrein has been shown to antagonize the contractile responses (Taha and Raza, 1998) of some agonists (Acetylcholine, adrenaline, nor adren-

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aline, oxytocin and prostaglandins) on different smooth muscle preparations dose dependently. However this effect was reversed by the increasing concentrations of Ca^{2+}, used in the nutrient medium. It has been shown that the antagonism produced by ambrein to some agonists was probably due to its interference with the mobilization of extracellular Ca^{2+} required for smooth muscle contractions induced by these agonists. Because of our continuing interest in this property, the present study was undertaken to investigate the effects of ambrein after its acute use in rats, on some cardiovascular parameters like heart rate, force of contraction and blood pressure and flutters, alone or before some agonist as well as antagonists. Part of stimulus for this investigative effort has been the problem that currently available agents for cardiac rhythm disorder are limited by serious toxicity. The development and clinical evaluation of safe, effective and long-lasting drug for the management of cardiac rhythm disorder presents an immediate challenge to the cardiovascular pharmacologists.

The present article has attempted to present the possible use of folkloric drug ambergris or ambrein, its pharmacology and therapeutic applications in cardiovascular ailments.

Experimental

Drugs and animals – Organic ambergris was purchased from a local market in Riyadh, Saudi Arabia. Ambrein was isolated and identified chemically by the authors (Taha 1989a; b). Other drugs used in this study were acetylcholine (ACh), adrenaline (AD), noradrenaline (NA), nicotine, isoprenaline, histamine (Fluka Chemie AG, Buchs, Switzerland), atropine sulphate (BDH, Poole, England), atenolol (Tenormin®, ICI, PLC, Macclesfield Cheshire, Great Britain) and verapamil (Orion Pharmaceutica, Espoo, Finland).

Male wistar albino rats roughly 10-12 weeks old weighing 200-225 g each, bread at Experimental Animal Care Centre, College of Pharmacy, King Saud University, Riyadh, were used in this study. The animals were housed at controlled temperature (22±1°C) and humidity. Food and water were made freely available.

Procedures – The rats were anaesthetized with ethylcarbamate (25% w/v aqueous solution) at a dose of 0.6 ml/100 g body weight, one hour before the experiment. The animal was placed on its back on a temperature controlled (37°C) operating table. The common carotid artery was located, cannulated and connected to a blood pressure sensitive transducer (ITT CANNON-wk-6-21C-¼) which was connected with a physiograph (Desk Model DMP-4B, Narcobiosystems Inc. Houston, Texas, USA). The venous cannula was inserted and tied into an external jugular vein. This cannula was connected to an injection block which allows injection of drug followed by saline (0.2 ml) to wash it all in. The animal also received heparin 0.1 ml (5000 units/ml). Fresh aqueous solutions of all drugs were prepared and injected intravenously (i.v.) into jugular vein, except ambrein which was solubilized in olive oil by gentle heating and was administered intraperitoneally (i.p.). In each case the dose volume was kept constant at 0.2 ml. A series of the trial doses of the selected drugs was administered to see their individual responses at certain dose levels. Then, the dose with appropriate response was selected for further experiments. In case of ambrein pretreatment ambrein was injected 60 min before the selected drug. For each drug a minimum of five experiments were performed. Preliminary experiments revealed that i.p. injection of vehicle (olive oil) at 0.2 ml dose had no significant effect on any of the parameters studied, when compared to the untreated control group. The dose of ambrein used in this study was in the range of the dose reported earlier to be pharmacologically active (Taha and Ginawi, 1993; Taha, 1994).

Statistical Analysis

The data are mean±standard error of means (SEM). Students *t*-test was used to determine the significant differences between the groups when compared to the control group.

Results

The results are shown in Fig. 1 and tables 1-2. Intraperitoneal administration of ambrein in the dose range of 50-200 mg/kg to male normotensive rats decreased the heart rate and force of contraction. These effects persisted for almost 1 hour of the ambrein treatment at the low dose (50 mg/kg). Myocardial contractility improved by 34% and 16% above the normal at 30 and 60 min time points respectively, which almost recovered to normal at 90 min time interval. This dose, had essentially no

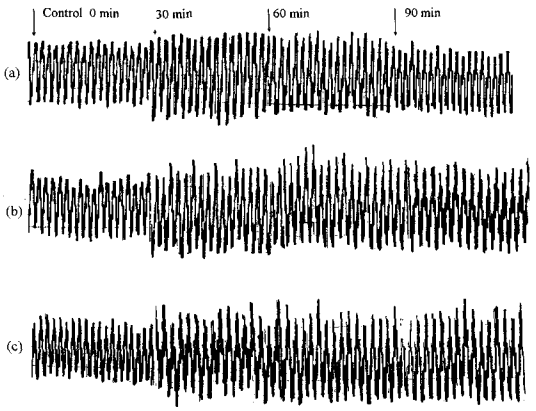

Fig. 1. Effect of ambrein treatment on heart rate, force of contraction and blood pressure at different time intervals. a) Ambrein 50 mg/kg ip, b) Ambrein 100 mg/kg ip, c) Ambrein 200 mg/kg ip

effects on heart rate and blood pressure of the animals. The 100 and 200 mg/kg doses affected the heart rate but not very significantly. The contractility improved very significantly ($P<0.01$) at all time intervals and was persistent throughout the study period (Table 1). All the acute doses of ambrein had essentially no effect on blood pressure of anaesthetized rats.

In the present study, isoprenaline 0.3 µg (a non selective ß-adrenoceptor agonist) produced positive chronotropic and inotropic effects accompanied by a decline in blood pressure. Dissimilar results were obtained with the ambrein pretreatment that inhibited any positive chronotropic effect to occur. However, positive inotropic effects were more pronounced with isoprenaline after ambrein pretreatment.

Adrenaline (0.5 µg) and noradrenaline (0.5 µg) administered to rats by slow i.v. injection caused the initiation of powerful cardiac reflexes, which are accompanied by elevations in blood pressure and an increase in frequency of heart rate, but to a lesser extent. The force of contraction was increased slightly significantly. While, the pretreatment with ambrein (100 mg/kg) did not antagonize the effect on blood pressure and heart rate. However, contractility was enhanced very significantly ($P<0.01$) showing additive effects of adrenomimetics.

After i.v. administration, the muscarinic effects of ACh predominated. At the dose of 0.5 µg (relatively low dose) ACh produced a fall in blood pressure and result in tachycardia. Pretreatment with ambrein, however, failed to avert the effects of ACh on heart rate and blood pressure. A single i.v. bolus of nicotine (25 µg) caused activation of the sympathoadrenal system resulting in the release of adrenaline and noradrenaline indicated by positive inotropic and chronotropic effects with a rapid increase in blood pressure. Ambrein pretreatment did not abolish these effects. However, these sympathoadrenal system stimulatory effects were counteracted significantly ($P<0.01$) and the force of contraction was further enhanced with nicotine after ambrein. Histamine

Table 1. Effect of ambrein pretreatment on heart rate, force of contraction and blood pressure changes induced by some cardioactive agents in anaesthetised rats

Group No.	Treatment	Without Ambrein Pretreatment			With Ambrein Pretreatment		
		Heart rate "beats/min"	Force of contraction "g"	B.P. "mmHg"	Heart rate "beats/min"	Force of contraction "g"	B.P. "mmHg"
1	Control	270±4.5	1.55±0.10	114±2.6	---	---	---
2	Ambrein	---	---	---	250±3.9**	2.32±0.18**	127±5.8
3	Acetylcholine	293±4.7**	1.68±0.21	86±3.9***	289±3.7	2.68±0.43*	110±4.7**
4	Adrenaline	315±5.8***	1.77±0.14	205±3.5***	268±5.3***	2.71±0.41*	176±6.9**
5	Noradrenaline	320±8.1***	1.83±0.18	182±3.8***	282±7.6**	2.95±0.37*	243±9.2***
6	Isoprenaline	324±7.5***	2.37±0.28*	90±3.7***	238±6.5***	3.14±0.21*	131±5.8***
7	Nicotine	301±6.3**	2.38±.23**	217±4.9***	270±4.5**	2.69±0.06	142±6.1***
8	Histamine	330±6.9***	1.73±0.16	95±4.3**	272±7.2**	2.28±0.13*	151±7.5***
9	Atropine	249±4.1**	1.39±0.42	101±2.1**	260±3.4	2.59±0.38*	108±4.4
10	Atenolol	247±5.7*	0.62±0.21**	88±2.1***	255±3.9	2.28±0.51*	130±3.7
11	Verapamil	143±7.8***	0.44±0.15***	42±3.6***	146±4.9	0.73±0.11	45±7.1

Pretreatment values in each group were compared to group 1. Group 2 was compared to group 1. Post-treatment values were statistically compared to pretreatment values in the same group (Students t-test).
*P<0.05, **P<0.01, ***P<0.001. Each value represents mean±S.E. of five observations.

Table 2. Effect of ambrein treatment on heart rate (H.R.), force of contraction (F.C.) and blood pressure (B.P.) of normotensive anaesthetized rats

Group No.	Treatment Dose	Post Treatment Time (min)											
		0:0			30			60			90		
		H.R. Beats/ min	F.C. g	B.P. mmHg	H.R. Beats/ min	F.C. g	B.P. mmHg	H.R. Beats/ min	F.C. g	B.P. mmHg	H.R. Beats/ min	F.C. g	B.P. mmHg
1	Control Olive oil 1ml/kg	270± 4.4	1.53± 0.98	121± 3.0	280± 5.5	1.56± 0.11	130± 5.4	278± 4.5	1.58± 0.10	120± 4.1	275± 9.1	1.58± 0.10	119± 5.3
2	Ambrein 50 mg/kg	265± 6.9	1.50± 0.10	112± 2.9	272± 4.5	1.95± 0.12*	134± 9.1	280± 4.7	1.69± 0.10	130± 4.2	297± 6.2	1.44± 0.09	120± 4.1
3	Ambrein 100 mg/kg	268± 4.7	1.58± 0.10	115± 2.6	270± 6.1	2.15± 0.11**	135± 4.3	245± 3.7***	2.29± 0.09***	128± 6.1	250± 4.0*	2.29± 0.12**	131± 5.6
4	Ambrein 200 mg/kg	276± 4.6	1.56± 0.11	119± 2.6	226± 5.7***	2.23± 0.12**	140± 4.9	176± 5.3***	2.45± 0.11***	141± 5.7*	175± 5.1***	2.53± 0.12***	139± 6.1*

Groups 2, 3 and 4 were statistically compared to group 1 at respective time points. Each reading is mean±S.E. of five observations.
*P < 0.05, **P < 0.01, ***P < 0.001 (Students t-test).

given i.v. showed a positive chronotropic effect at the acute dose (0.6 μg). The blood pressure of the anaesthetized rat declined slightly but insignificantly. Ambrein pretreatment totally inhibited the chronotropic and blood pressure changes. On the other hand, the increased contractility with ambrein pretreatment remained unaffected by histamine.

Atropine a muscarinic blocking agent, produced a slight decrease in heart rate following i.v. administration of a single low dose (200 μg). A decrease in blood pressure followed by arrhythmia was observed. Ambrein pretreatment did not reverse the condition except an increase in contractility of heart.

Atenolol (100 μg), a selective $ß_1$ antagonist, when given alone to anaesthetized rats decreased the heart rate, myocardial contractility and blood pressure highly significantly. Pretreatment with single dose of ambrein totally abolished the said effects. The effect of ambrein on the force of contraction was significantly obvious and persistent.

Verapamil, a depressant of the slow inward current of sinus node resulted in negative chronotropy, a significant negative inotropy and prompt hypotension after a single acute i.v. 50 μg dose. However, ambrein pretreatment intensified the effects of verapamil (Table 2).

Discussion

The heart expresses both $ß_1$ and $ß_2$ adrenoceptors and stimulation of either of these receptor subtypes can mediate increase in heart rate (Hall *et al.*, 1989) and contractility (Gille *et al.*, 1985; Zerkowski *et al.*, 1986; Kaumann and Lemoine, 1987; Lemoine *et al.*, 1988). However, only $ß_1$ receptors mediate chronotropic and inotropic responses in rat heart (Wilson and Lincoln, 1984; Molenaar and Summers, 1987). Although $ß_1$ receptors mediated responses predominate but it has also been shown that stimulation of $ß_2$ adrenoceptors increased the heart rate in man (Arnold *et al.*, 1985; Gille *et al.*, 1985; Kaumann and Lemoine, 1987) and $ß_2$ selective drugs affect chronotropy more than inotropy (Carlson *et al.*, 1977; Stiles *et al*, 1983; Friedman *et al.*, 1987). The present results on ambrein alone have been shown to induce negative chronotropic and very significant positive inotropic responses. All doses of ambrein induced markedly significant effects. The reduction in heart rate and increase in contractility which was observed, occurred in a dose-dependent-manner in both the high doses but the lowest dose did not change the heart rate. However, at any of the dose tested no significant change in blood pressure occurred.

Other researchers have observed that ß-adrenoceptor agonists (catecholamines like adrenaline, noradrenaline and isoprenaline) induce positive chronotropic and inotropic effects in animal hearts (Arnold *et al.*, 1985; Stiles *et al.*, 1983; Friedman *et al.*, 1987). In the present study all of these non selective ß-adrenoceptor agonists markedly increased the heart rate and contractility at the doses tested, when used alone. Pretreatment with ambrein at 100 mg/kg dose

averted the effects of isoprenaline completely but did not antagonize the effects on blood pressure. A slight decrease in blood pressure was observed when catecholamines were administered after ambrein as compared to catecholamines alone. However, myocardial contractility was further enhanced with adrenaline, noradrenaline and isoprenaline after an elevation with ambrein pretreatment.

Acetylcholine given systemically causes a fall in blood pressure owing to generalized vasodilatation and follows reflex tachycardia. The dilatation of vasculature is reported to be due to presence of muscarinic receptors located in the endothelial cells of vascular bed. By the stimulation of these receptors an endothelium-derived relaxing factor is released causing the adjacent smooth muscle cells to relax (Furchgott and Zawadzki, 1980; Vanhoutte, 1989) and result in hypotension; but compensatory tachycardia occurs. These effects may arise from inhibition of noradrenaline from sympathetic (adrenergic) nerve endings by ACh. These effects induce negative inotropy, and, at least partly, are due to inhibitory effects of muscarinic agonists on adenylcyclase activity (Watanabe, 1983). However, ambrein pretreatment did not augment any change in the response of ACh except a mild increase in contractility.

The cardiovascular responses to the systemically administered nicotine cause the stimulation of sympathetic ganglia and adrenal medulla following discharge of catecholamines from sympathetic nerve endings. It also result in sympathomimetic response to nicotine with a net result in the activation of chemoreceptors of aorta and carotid bodies and reflexly show vasoconstriction, tachycardia and elevation in blood pressure (Benowitz, 1986; Benowitz *et al.*, 1988; Russel and Feyerabend, 1978). Our results are in agreement with these observations. However, ambrein given as a pretreatment, almost totally blocked the sympathoadrenal effects with an additive response in contractility that was enhanced with ambrein pretreatment.

It is evident that histamine functions as a neurotransmitter. Histamine containing neurons may regulate many functions like drinking, thermoregulation of body, antidiuretic hormone secretions, perception of pain and blood pressure (Hough, 1988). The effect of histamine i.v. administration was a slight fall in blood pressure. Both H_1 and H_2 receptors seem to be involved in this response. In smooth muscle, activation of either type of receptors can elicit vasodilatation. H_1 receptors have high affinity for histamine and mediate a dilator response with a short lived rapid fall in blood pressure. Ambrein pretreatment totally blocked any of the changes in blood pressure and heart rate. However, the myocardial contractility that was increased after ambrein remained unaffected by histamine. In some previous studies regarding inflammation and oedema, ambrein was found to inhibit the histaminic responses (Taha, 1992; Taha and Ginawi, 1993), that also favours the idea of blockade of histamine release and antagonism to histaminic receptors in tissue and CNS.

Atropine a competitive antagonist of ACh and other muscarinic agonists, compete for a common binding site on muscarinic receptors (Yamamura and Snyder, 1974; Hulme *et al.*, 1978). Various muscarinic receptors regulate several effector systems with in the cells. Heart contain M_2 receptors (Rattan and Goyal, 1988). M_2 muscarinic receptors regulate ion channels (e.g. enhancement of K^+ conductance in cardiac atrial fibres) and inhibit adenylyl cyclase by interaction with a G protein. Literature has also suggested that the decreased heart rate by atropine is produced through blockade of M_1 receptors on postganglionic parasympathetic neurons and relieves inhibitory effects of synaptic ACh on the release of transmitter (Wellstein and Pitschner, 1988). Ambrein pretreatment failed to show any change in heart rate or blood pressure after atropine except a significant increase in contractility that was induced by ambrein.

ß-Adrenoceptor blocking agents are well known for their ability to reduce acutely cardiac function in both normotensive and hypertensive animals (Nles *et al.*, 1973; Garvey and Ram, 1975; Buckingham and Hamilton, 1980; Cohn, 1983) and in man (Ulryeh *et al.*, 1968; Pirchard and Gillam, 1969). This reduction in cardiac function has been shown to be with an increase in cardiac dimensions (Chamberlain, 1966; Helfant *et al.*, 1971). However, ß-adrenoceptor blockade results in a considerable fall in heart rate. The present results with atenolol, are in agreement with several studies performed on animals (Stiles *et al.*, 1983; Arnold *et al.*, 1985; Friedman *et al.*, 1987). In the present study ambrein pretreatment totally abolished the effects produced by atenolol alone. The effect of ambrein on force of contraction were significantly obvious and persistent.

Membrane depolarization in atrial and ventricular conducting tissue and in myocytes of the atria and ventricles is caused by Na^+ through the fast channel

and by Ca^{2+} through the slow channel (Coraboeuf, 1978). This depolarization (of sinoatrial and atrioventricular nodes) is mainly dependent on the movement of Ca^{2+} through the slow channel. In the myocardial contraction process Ca^{2+} is required to bind with troponin, relieving contractile apparatus to function. The blockade of slow channel by Ca^{2+} channel blockers can lead to negative inotropic effects. Verapamil not only reduces the magnitude of Ca^{2+} current through the slow channel but also decreases the rate of recovery of the channel (Ehara and Kaufmann, 1978). Pretreatment with ambrein caused an elevation in myocardial contractility. Verapamil after ambrein reduced the heart rate and force of contraction highly significantly. It also reduced the blood pressure. However, the negative inotropic and chronotropic responses were more intensive after ambrein pretreatment as compared to verapamil alone.

In conclusion the results of the present study show that the compound ambrein produced a decrease in heart rate and a little change in blood pressure in the anaesthetized normotensive rats. Also, this compound induced a considerable increase in force of myocardial contraction when used alone or with some other agonists or antagonists. The results on blood pressure did not show a numerable change in normotensive rats. The studies on the hypertensive disorders in animal models may define those variables which may contribute to these effects of ambrein on cardiovascular system. The mediation of neuromediators is reported to be possible and noradrenergic neurons in the CNS have projections to cholinergic, serotonergic and GABA-ergic components with a possible modulatory interaction between various neurotransmitter systems in the observed actions. However, the relevance of these actions of ambrein in cardiovascular responses remains to be investigated.

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