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TOPIC HIGHLIGHT

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Hypertension and chronic ethanol consumption: What do we know after a century of study?

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Abstract

The influences of life habits on the cardiovascular system may have important implications for public health, as cardiovascular diseases are among the leading causes of shorter life expectancy worldwide. A link between excessive ethyl alcohol (ethanol) consumption and arterial hypertension was first suggested early last century. Since then, this proposition has received considerable attention. Support for the concept of ethanol as a cause of hypertension derives from several epidemiologic studies demonstrating that in the general population, increased blood pressure is significantly correlated with ethanol consumption. Although the link between ethanol consumption and hypertension is well established, the mechanism through which ethanol increases blood pressure remains elusive. Possible mechanisms underlying ethanol-induced hypertension were proposed based on clinical and experimental observations. These mechanisms include an increase in sympathetic nervous system activity, stimulation of the renin-angiotensin-aldosterone system, an increase of intracellular Ca^{2+} in vascular smooth muscle, increased oxidative stress and endothelial dysfunction. The present report reviews the relationship between ethanol intake and hypertension and highlights some mechanisms underlying this response. These issues are of interest for the public health, as ethanol consumption contributes to blood pressure elevation in the population.

Key words: Ethanol; Hypertension; Calcium; Nitric oxide; Oxidative stress

Core tip: After a century of study, the relationship between chronic ethanol consumption and hypertension is well established. This review provides a description of the main studies that showed a relationship between chronic ethanol consumption and hypertension in humans. We also discuss studies using animal models of ethanol-induced hypertension, describing the main mechanisms by which ethanol consumption leads to hypertension.

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INTRODUCTION

Hypertension is a major independent risk factor for cardiovascular disease. In ethanol-consuming populations, the amount of ethanol consumed has a significant impact on blood pressure values, the prevalence of hypertension, and cardiovascular and all-cause mortality. The observa-

Table 1 List of the main epidemiological studies describing the relationship between ethanol consumption and hypertension

Ref.	Yr	Study	Subjects	Age (yr)
Lian[1]	1915		150	42-43
Clark *et al*[2]	1967	Los angeles heart	865	21[1]
Gyntelberg *et al*[3]	1974	Copenhagen	5249	40-59
Klatsky *et al*[4]	1977	Kaiser-Permanente Ⅰ	83947	15-79
Dyer *et al*[5]	1977	Chicago W. Electric	1899	40-55
Arkwright *et al*[6]	1982	Perth	491	20-45
Milon *et al*[7]	1982	Lyon	1134	20-59
Klatsky *et al*[10]	1986	Kaiser-Permanente Ⅱ	66510	-

[1]Mean age.

tion that the excessive consumption of ethyl alcohol (ethanol) is associated with high blood pressure is nearing its centennial mark[1]. In the last century, numerous epidemiologic studies have found an association between ethanol consumption and arterial hypertension[2-6]. It is estimated that 5% to 24% of hypertension cases are associated with ethanol consumption[7,8]. However, although the link between ethanol consumption and arterial hypertension is well established, the mechanism through which ethanol increases blood pressure remains elusive. The effects of ethanol on the cardiovascular system are complex, and attempts to evaluate the possible mechanisms underlying ethanol-induced hypertension in humans are hindered by several limitations. These difficulties include differences in the duration of ethanol use, the timing and frequency of blood pressure measurements, variability in the type and frequency of ethanol intake, age, gender, ethnicity, salt use, body mass index and comorbid conditions.

Animal models of alcoholism may be relevant to understanding the mechanisms by which ethanol consumption increases blood pressure. Data support the involvement of increased sympathetic activity, stimulation of the renin-angiotensin-aldosterone system, increased intracellular Ca^{2+} in smooth muscle with a subsequent increase in vascular reactivity, oxidative stress and endothelial dysfunction. In this review, we will discuss the relationship between ethanol intake and hypertension and some of the possible mechanisms underlying this response. For the present review, a MEDLINE-based search was conducted using the following keywords: "alcohol", "alcoholism", "ethanol", "blood pressure", "hypertension", "nitric oxide", "oxidative stress", "calcium", "endothelial dysfunction" and "vascular reactivity". Articles were further limited to those published in English (except the classic article published in French by Camille Lian) and containing abstracts. Reasons for the exclusion of articles include unclear ethanol dose or ingestion period. Information analysis started with the title, followed by the abstract and, finally, the complete report.

ETHANOL CONSUMPTION AND HYPERTENSION IN HUMANS (TABLE 1)

In 1915, the French army physician Camille Lian studied approximately 150 French career soldiers (42 and 43 years old), relating their drinking to high blood pressure. The results of this study showed a clear threshold relationship of heavy drinking to hypertension, which was defined as 150/100 mmHg, and very heavy drinking increased the risk further. The moderate drinkers consumed 2 L of wine per day, the heavy drinkers consumed more than 2 L per day, and the very heavy drinkers consumed 3 or more liters per day. This was the first report on this relationship, but the result was ignored for approximately 50 years. In the 1960s and 1970s, findings among smaller patient populations corroborated the initial results described by Lian[2,3].

In this review, for the purpose of standardization, the levels of ethanol consumption in humans have been expressed as the number of standard drinks per day (1 standard drink is defined here as the equivalent of 14 g of ethanol). A landmark observational study published in 1977, the Kaiser-Permanente Multiphasic Health Examination Data, reported differences in systolic blood pressure as high as 11 mmHg in individuals consuming 6 or more drinks per day compared with non-drinkers[4]. This study was based on self-administered questionnaires from more than 80000 men and women and showed that a threshold of 3 or more drinks per day was a risk factor for hypertension across races and in both sexes. Moreover, the study found a relationship between the amount of ethanol consumed and blood pressure. This observation was corroborated by other studies. For example, among Danish men aged 40-59 years, the differences in blood pressure between those consuming 6 or more drinks per day and those consuming fewer drinks per day were 8 mmHg (systolic) and 4.5 mmHg (diastolic)[3]. Systolic pressure increased progressively with increasing ethanol consumption among 491 Caucasian males aged 20-45 years. Importantly, the effect of ethanol on systolic blood pressure was independent of the effects of age, obesity, cigarette smoking and physical activity[9].

The second Kaiser-Permanente study reconfirmed the relationship of higher blood pressure to ethanol use[10]. Data from approximately 80000 persons, collected in the United States from 1978 to 1981, revealed a direct positive relationship between the regular consumption of alcoholic beverages and higher blood pressure, independent of potential confounding factors, including age, body weight and smoking status. One important finding of this study was that at 1 to 2 drinks per day, there was a slight but significant increase in blood pressure, indicating that the threshold was lower than that reported in the first Kaiser-Permanente study. The change in the threshold values between the two studies was the result of the division of lighter drinkers into several categories in the second study. As observed previously in the first Kaiser-Permanente study, systolic and diastolic blood pressures substantially increased at 3 to 5 and 6 or more drinks per day.

In his review of studies examining the prevalence of hypertension in ethanol consumption groups, MacMahon (1987) analyzed 29 cross-sectional studies and 6 prospec-

tive studies conducted in populations from a variety of geographic regions, including North America, Australia, Japan, Europe and New Zealand. Most of these studies reported a significant positive association between hypertension and ethanol consumption[11]. The association was shown to be independent of confounders such as age, body mass index, smoking status and exercise. In general, the studies highlighted that the increase in systolic pressure was greater than that in diastolic pressure and that there was a trend toward a greater effect of ethanol on blood pressure in older men compared with younger men. Finally, the studies showed that at 3 to 4 drinks per day, the prevalence of hypertension was approximately 50% greater than that in non-drinkers, and at 6 to 7 drinks per day, the prevalence was 100% greater.

The exact threshold for the effect of ethanol on blood pressure is not clear. In fact, the threshold question is controversial, as epidemiologic studies could not resolve the question of a possible threshold for the apparent risk of hypertension. While several studies have suggested little or no effect of up to 1 or 2 drinks per day on blood pressure[2-4,12], others have shown a progressive linear association[6,7,13]. The first Kaiser-Permanente study described a threshold relationship at 3 to 5 drinks a day for men, with a substantial increase in systolic blood pressure at 6 drinks a day[4]. However, the threshold was found to be at a much lower drinking level than that described in the first Kaiser-Permanente study. Significantly higher systolic pressures were found in Caucasian males who consumed 2 or fewer drinks a day[9]. The second Kaiser-Permanente study described that at 1-2 drinks per day, there was a slight but significant increase in blood pressure[4]. A slight increase in blood pressure was found in men reporting as few as 1 to 2 drinks per day in that survey.

The contribution of ethanol consumption to the prevalence of hypertension is dependent upon the population studied and varies widely in different populations. In developed countries such as the United States and England, it has been estimated that as much as 30% of hypertension may be attributed to ethanol consumption[14]. Other studies suggested this proportion to be smaller. The Australian Risk Factor Prevalence Study[15] estimated that 7% of the prevalence of hypertension could be attributed to ethanol consumption, while the first Kaiser Permanente Study estimated a proportion of 5%[4]. In these two studies, it was estimated that a maximum of 11% of hypertension in men could be attributed to the consumption of ethanol. A French epidemiological study estimated that 24% of the prevalence of hypertension in French men could be attributed to ethanol consumption[7]. Similar results were found in a cross-sectional study in Sidney, where it was estimated that 24% of hypertension may be attributed to ethanol consumption[16].

The estimate is somewhat lower in women and higher in men[4,10]. In the Risk Factor Prevalence Study[15], ethanol consumption accounted for no more than 1% of hypertension in women. The reasons for the gender difference in the proportion of hypertension prevalence associated with ethanol consumption are not fully understood, but they are most likely attributed to the less consumption of ethanol by women than men[11].

The mechanism(s) by which ethanol consumption leads to elevations in blood pressure is uncertain. A small number of studies in humans have attempted to address this question. The role of catecholamines in mediating the effects of ethanol on blood pressure has been investigated in humans. In this regard, increases in plasma adrenaline[17] and noradrenaline[18] were described in humans after ethanol ingestion, and it was suggested that activation of the adrenergic system may be responsible for the increased blood pressure. On the other hand, Potter *et al*[19] did not observe changes in catecholamines levels after ethanol consumption. Moreover, these authors reported that plasma renin and cortisol levels were not affected by the consumption of ethanol[19]. Arkwright *et al*[9] observed that, although blood pressure was higher among ethanol drinkers, there were no changes in plasma adrenaline, noradrenaline, cortisol and renin in these subjects. Conversely, Ibsen *et al*[20] showed increased plasma renin levels among heavy ethanol drinkers. Potter *et al*[19] found that plasma cortisol, but not plasma rennin, increased after ethanol consumption. The reason for the inconsistencies among these results is uncertain, and further studies on the mechanisms underlying the pressor effects of ethanol in humans would be of value. The results of these studies raise a number of possibilities concerning the involvement of humoral mechanisms in the pressor effects of ethanol. However, the available data in humans are not sufficient to allow substantive conclusions. In light of the need for careful investigation of the mechanisms underlying the effects of ethanol on blood pressure, experimental models were created and are used for this purpose.

ANIMAL MODELS OF ETHANOL-INDUCED HYPERTENSION

Most experimental studies corroborate the findings of the epidemiological studies described above, confirming that ethanol consumption is associated with increased blood pressure levels and an increased prevalence of hypertension. Chan and Sutter[21] found that treatment of male Wistar rats for 12 wk with a solution of ethanol (20% *v*/*v*) resulted in mild hypertension. An increase of approximately 25% in mean arterial blood pressure (from 98 to 122 mmHg) was described later by these authors using the same experimental model[22]. Similarly, Abdel-Rahman *et al*[23] observed an increase in systolic blood pressure after 12 wk of ethanol feeding (20% *v*/*v*) in Wistar and Sprague-Dawley rats. Blood pressure was significantly higher at week 6 in Sprague-Dawley ethanol-fed rats (from 106 to 147 mmHg) and at week 8 in Wistar ethanol-fed rats (from 117 to 149 mmHg). The authors also found that ethanol-fed rats had a higher sympathetic activity, as beta-blockade with propranolol decreased heart rate to

a greater degree in ethanol-fed rats than it did in control rats[23]. Strickland and Wooles[24] showed that the systolic and diastolic pressures of ethanol-fed (ethanol 20% *v/v*) Sprague-Dawley rats became significantly greater at 4 wk and continued to increase throughout the remainder the study. The systolic blood pressure of ethanol-fed rats was increased by 6.6 mmHg at 4 wk and by 33.8 mmHg at 22 wk compared with the controls. The difference in diastolic blood pressure between the control and ethanol-fed rats was 5.8 mmHg at 4 wk, and this difference increased to 47 mmHg by 22 wk of ethanol feeding[24]. Vasdev *et al*[25-27] described an increase in systolic blood pressure in male Wistar rats after 1 wk of treatment with ethanol. The rats were given 5% ethanol in their drinking water for 7 wk, and the systolic blood pressure in the ethanol-treated rats was found to be significantly higher than that in the controls after 1 wk or longer[25-27]. Interestingly, the discontinuation of ethanol treatment for 7 wk did not reverse the hypertension or the adverse renal vascular changes in ethanol-induced hypertensive rats[25].

In the study of Utkan *et al*[28], systolic blood pressure was recorded weekly using the tail-cuff method in Wistar rats treated with ethanol (7.2% *v/v*) for 4 wk. There was a mild but significant elevation of systolic blood pressure in the ethanol-fed rats by week 1 compared to baseline measurements, and this difference remained higher at later times. This study showed that the hypertensive state associated with ethanol intake can be observed in the early stages of ethanol consumption. A possible explanation for such a finding could be the higher blood ethanol levels found in this study (293.6 ± 5.2 mg/dL)[28]. Brown *et al*[29] showed that ethanol-consuming Sprague-Dawley rats exhibited elevated systolic blood pressures compared with the control group (151.6 ± 0.6 *vs* 132.9 ± 2.7 mmHg). In this study, the blood ethanol levels averaged 63.8 ± 2.5 mg/dL.

In a previous study, we compared the effects of ethanol intake (20% *v/v*) for 2, 6 and 10 wk on arterial blood pressure in conscious Wistar rats[30]. The baseline systolic, diastolic and mean arterial pressure values of ethanol-treated rats were increased (approximately 20%) after the 3 different periods of treatment. Because blood pressure was already elevated in the 2-wk-treated rats, our results supported the notion that the hypertensive state associated with ethanol intake can occur in the early stages of ethanol consumption. This finding contrasted those of previous studies, which have reported that blood pressure elevation occurred late during chronic ethanol treatment[23,24,28]. Blood ethanol content is a potential explanation for the disparity among reports.

Using this same model of ethanol feeding, we investigated the effects of ethanol treatment for 2 and 6 wk on both blood pressure and vessel reactivity. Mild hypertension was observed in chronically ethanol-treated rats, which was due to increases in both systolic and diastolic pressures. Chronic ethanol consumption in rats increased the contractile response of the aorta and mesenteric arterial bed[31-33]. In addition to its hypertensive effect, ethanol consumption can also modulate the response to vasoactive agents *in vivo*. Data from our group showed that chronic ethanol consumption increased blood pressure as well as the pressor response induced by phenylephrine and endothelin-1[30,34].

The studies using animal models established a positive correlation between the duration of ethanol consumption and the increase in blood pressure, showing that the period of exposure to ethanol is an important factor in the development of hypertension[23,24]. Additionally, there is evidence that blood ethanol concentration contributes to the increase in blood pressure in animal models of alcoholism, where higher blood ethanol concentrations may account for the earlier development of hypertension. Previously, we showed that increased blood pressure, concomitant with ethanol feeding, was observed in 2-wk ethanol-treated animals, in which the blood ethanol content was 1.67 ± 0.21 mg/mL[30]. Abdel-Rahman *et al*[23] reported a blood ethanol concentration of 0.53 ± 0.04 mg/mL in 12-wk-treated rats. Additionally, Abdel-Rahman *et al*[23] (1985), who did not detect blood pressure changes after ethanol treatment, reported a blood ethanol concentration of 0.34 ± 0.04 mg/mL in rats treated with ethanol for 30 d[35].

Several mechanisms have been postulated for the hypertensive response to chronic ethanol consumption. Evidence suggests the existence of a myogenic mechanism(s) that involves alterations in the contractile/relaxant properties of vascular smooth muscle. In fact, the majority of studies describing the effects of ethanol on arterial blood pressure also evaluated the effects of ethanol on vascular responsiveness[24,28,29,31-33].

MECHANISMS UNDERLYING ETHANOL-INDUCED HYPERTENSION (TABLE 2)

Myogenic mechanism

Much of the research investigating the chronic effects of ethanol on the cardiovascular system has addressed vascular responsiveness to vasoconstrictor agents. In this regard, enhanced vascular reactivity to vasoconstrictor agents or impairment of vascular relaxation is described to contribute to the cardiovascular complications associated with chronic ethanol consumption. The initial studies in this field showed enhanced vascular reactivity to α_1-adrenoceptor agonists in different arteries from ethanol-fed rats. Pinardi *et al*[36] found that chronic ethanol consumption significantly enhanced the contractile response induced by phenylephrine of endothelium-intact aortic rings. Noradrenaline-induced contraction of the superior mesenteric artery was shown to be greater in rings from ethanol-treated rats[37]. Likewise, there was an ethanol-associated increase in the maximal contractile response to phenylephrine, a selective α_1-adrenoceptor agonist, in endothelium-denuded aortic rings[38]. Later, Ladipo *et al*[39] demonstrated that chronic ethanol consumption increased the sensitivity of rat aortic rings to noradrenaline. At this point, although it was well estab-

Table 2 Summary of the main mechanisms underlying ethanol-induced hypertension

Ref.	Mechanism
[17,18]	Increase in sympathetic nervous system activity
[20]	Stimulation of the renin-angiotensin-aldosterone system
	Myogenic mechanism:
[31,32,36-42]	Enhanced vascular reactivity to vasoconstrictor agents
[33,41,44-46]	Impairment of the vascular relaxation
	Oxidative stress:
[70-77]	Increase in reactive oxygen species generation
[81,82,85-87]	Reduction of antioxidant systems
[28,44,52,95-102]	Decrease of nitric oxide bioavailability and endothelial dysfunction

lished that chronic ethanol consumption enhanced α_1-induced contraction, the mechanisms underlying this response were poorly understood. Moreover, the experiments designed to study the vascular effects of chronic ethanol consumption on α_1-induced contraction used only one period of treatment[21,28,29]. Based on these observations, we proposed a study to investigate the time-course of changes in vascular reactivity to phenylephrine in aortas from chronically ethanol-treated rats as well as to evaluate in detail the mechanisms underlying the effects of long-term ethanol consumption on α_1-induced contraction. Chronic ethanol consumption produced an increased responsiveness to phenylephrine in aortas, although there was no relationship between the period of treatment (2, 6 and 10 wk) and the magnitude of the enhancement of α_1-induced contraction[40]. Importantly, the increased responsiveness to phenylephrine was also observed after endothelial denudation, further suggesting that the increased sensitivity to α_1-adrenergic agonists was not dependent on the presence of the endothelium. The enhanced vascular response to phenylephrine observed in the aorta of ethanol-treated rats was maintained by two mechanisms: an increased release of thromboxane A2, a vascular smooth muscle-derived vasoconstrictor prostanoid, and an increased extracellular Ca^{2+} influx. One interesting finding of this study was that the increased response to phenylephrine was not the result of a nonspecific increase in rat aorta reactivity induced by chronic ethanol intake, as the contractile responses to endothelin-1 or KCl were not affected by the ethanol treatment. In fact, while studying the effect of ethanol consumption on the reactivity of rat carotids to endothelin-1, we found an increase in endothelin-1-induced contraction in this artery with no change in the contraction induced by phenylephrine[41,42]. The hyperactivity to endothelin-1 in the rat carotid was not different among the three periods of treatment (2, 6 and 10 wk) used in our study. The potentiation of endothelin-1-induced contraction in the rat carotid was caused by reduced expression of pro-relaxation endothelial endothelin receptor type B (ET_B) receptors.

Most of the experiments designed to study the relationship between alterations in vascular functionality and increases in blood pressure induced by ethanol consumption used conduit vessels, such as the aorta. However, while the aorta does not offer substantial resistance to blood flow, the contribution made by vessels of smaller diameter to peripheral vascular resistance is much greater. In rats, the mesenteric circulation receives approximately one-fifth of the cardiac output[43], and thus, regulation of this bed provides a significant contribution to the regulation of systemic blood pressure. To further analyze this aspect, we evaluated whether alterations in the reactivity of the mesenteric arterial bed could account for the hypertensive state associated with ethanol consumption[31]. Chronic ethanol consumption produced an endothelium-dependent increased responsiveness to phenylephrine in a perfused mesenteric arterial bed isolated from rats treated with ethanol for 6 wk but not from rats treated for 2 wk. However, increased blood pressure was observed in ethanol-treated animals after 2 wk, whereas altered responsiveness to phenylephrine was only observed in rats treated for 6 wk. These observations supported the notion that the altered responsiveness of resistance arteries was not the cause, but rather the consequence, of the increased blood pressure associated with ethanol intake[31,32]. The increased vascular response to phenylephrine observed in the mesenteric arterial bed was maintained by two mechanisms: an increased release of endothelial-derived vasoconstrictor prostanoids and a reduced modulatory action of endothelial nitric oxide (NO); the latter is likely associated with a reduced expression of the enzyme eNOS (endothelial NO synthase)[32].

Impairment of vascular relaxation may also contribute to the cardiovascular complications associated with chronic ethanol consumption. Long-term ethanol consumption significantly reduced acetylcholine-induced relaxation in the aortic rings from rats treated with ethanol for 12 wk[44] and 8 wk[45]. In the rat carotid, the relaxation induced by IRL1620, a selective endothelin ET_B receptor agonist, was reduced after treatment with ethanol; this effect was mediated by a mechanism involving the down-regulation of endothelial ET_B receptors[41]. More recently, we found that chronic ethanol consumption reduced the endothelium-dependent relaxation induced by the peptide adrenomedullin in the rat aorta[46].

In resistance arteries, Hatton *et al*[37] showed an increased response of mesenteric arteries to noradrenaline in rats treated with ethanol for 18 wk. The finding that the relaxation induced by acetylcholine, but not by sodium nitroprusside, was reduced in the mesenteric arterial bed from ethanol-treated rats indicated that chronic ethanol consumption decreased the action of NO or its endothelial cell receptor-stimulated production/release[32]. Similarly, ethanol consumption was also found to reduce the endothelium-dependent relaxation induced by adrenomedullin in the rat mesenteric arterial bed[33]. The vascular relaxation induced by adrenomedullin in the rat mesenteric arterial bed is endothelium-dependent and involves the activation of the NO-cyclic guanosine monophosphate pathway[47]. In our study, no differences in adrenomedullin-induced relaxation were detected in control and ethanol-exposed tissues after incubation with

L-nitro-arginine methyl ester, a NOS inhibitor, suggesting that the reduced adrenomedullin responsiveness of the mesenteric arterial bed from ethanol-treated rats was due to an impaired modulation of adrenomedullin-induced relaxation by NO[33].

The vascular endothelium and vascular smooth muscle cells are important targets for the effects of ethanol consumption. These effects are complex, and the identification of biochemical/molecular mechanisms that could explain such effects is warranted. A number of mechanisms have been postulated to explain the pathogenesis of high-dose ethanol toxicity in the vasculature. These mechanisms include an increase in intracellular Ca^{2+} levels with a subsequent increase in vascular reactivity, oxidative stress and a reduction in NO bioavailability. These processes will be discussed in the following sections.

Alterations in Ca^{2+} levels

One of the mechanisms by which chronic ethanol consumption leads to alterations in vascular responsiveness is by increasing the intracellular Ca^{2+} levels in vascular smooth muscle cells. Ca^{2+} is a cation of critical importance for many cellular control mechanisms, including muscle contraction. During excitation, the intracellular Ca^{2+} concentration increase by either (1) Ca^{2+} entry through the plasma membrane through voltage- or ligand-gated ion channels, or (2) release from intracellular stores (sarcoplasmic reticulum or mitochondria).

Some studies have provided evidence that ethanol consumption increases the intracellular Ca^{2+} concentration. This response may result from a direct effect of ethanol on plasma membrane permeability, Na^+ transport and Na^+-Ca^{2+} exchange, and/or impaired Ca^{2+} transport due to a secondary abnormality, such as Mg^{2+} depletion, which is described in alcoholics[48]. Increased Ca^{2+} influx results in increased vascular contractility and reactivity, and those responses increase vascular tone and peripheral vascular resistance, thereby elevating blood pressure[49]. Tirapelli *et al*[40] described an increased phenylephrine-induced contractility of arteries from ethanol-treated rats. SQ29548, a potent and selective thromboxane A2 receptor antagonist, reduced the maximal $CaCl_2$ response of aortic rings from ethanol-treated rats, suggesting that the enhanced response to extracellular Ca^{2+} was modulated by PGH_2/TXA_2. Based on these results, it was concluded that prostanoids mediate the enhanced reactivity to phenylephrine by mechanisms that alter the mobilization of or sensitivity to extracellular Ca^{2+}[40].

The effect of chronic ethanol administration on blood pressure and its relation to Ca^{2+} were also investigated by Hsieh *et al*[50] in 7-wk-old Wistar rats that had received 15% ethanol in their drinking water. The blood pressure in ethanol-treated rats was significantly higher than in the controls. The extracellular fluid volume was increased in ethanol-treated rats, and the blood pressure significantly correlated with increases in the intracellular Ca^{2+} concentration. These results suggest that increased intracellular Ca^{2+} and augmented body fluid volume contributed to the development of ethanol-induced hypertension. It was also suggested that these responses were partly mediated by Mg^{2+} depletion and suppressed Na^+ pump activity[50]. In fact, these factors appear to be all-important in the etiology of hypertension[51].

In 2008, Tirapelli *et al*[52] reported an increased responsiveness to KCl of arteries from female rats chronically treated with ethanol. Because KCl-induced contraction depends almost exclusively on Ca^{2+} influx through the activation of voltage-sensitive channels[53], it was suggested that ethanol consumption increases the Ca^{2+} influx through these channels. Vasdev *et al*[54] observed that ethanol consumption (10% ethanol in drinking water-6 wk) increased systolic blood pressure and that this response was associated with an increased Ca^{2+} uptake by aortas from ethanol-treated animals. These findings suggested that increases in cytosolic free Ca^{2+} and in Ca^{2+} uptake in the vasculature are associated with ethanol-induced hypertension. Two years later, these authors reported that verapamil, a Ca^{2+} channel blocker, reversed the increase in systolic blood pressure and aortic Ca^{2+} uptake induced by chronic ethanol consumption. In addition to the effects observed previously, the authors observed smooth muscle cell hyperplasia in small arteries and in renal arterioles from ethanol-treated rats[25].

In a clinical study, it was demonstrated that both systolic and diastolic blood pressures were significantly higher in individuals drinking 275 g ethanol per week[55]. In these subjects, increased plasma Ca^{2+} levels were correlated with increased diastolic blood pressure. An increment in diastolic pressure of 6.9 mmHg correlated with increments of 0.1 mmol/L in plasma Ca^{2+} concentration. Those findings suggested that regular ethanol consumption predisposes to hypertension by facilitating Ca^{2+} accumulation in cells involved in blood pressure regulation[55]. Taken together, the above-mentioned studies suggest a role for Ca^{2+} in ethanol-induced hypertension. In this scenario, ethanol consumption would alter Ca^{2+} influx/permeability in the vasculature with a consequent increase in vascular contractility and peripheral resistance, which in turn would be responsible for the increase in blood pressure associated with ethanol consumption.

Oxidative stress

Reactive oxygen species (ROS) are reactive chemical entities produced as intermediates in reduction-oxidation (redox) reactions. Perturbations of the balance between ROS production and scavenging by antioxidant systems result in oxidative stress and presumably in pathophysiologic changes. Oxidative stress is a common mediator of pathogenicity in cardiovascular diseases, such as hypertension[56,57]. ROS have an important pathophysiological role in inflammation (by influencing platelet aggregation and migration of monocytes), hypertrophy, proliferation, fibrosis, angiogenesis, processes that are involved in cardiovascular remodeling and endothelial dysfunction[58-61].

The role of ROS in the pathophysiology of hypertension is well established[62-64]. The causal relationship between ethanol, ROS and hypertension most likely occurs at the vascular level, where ethanol promotes oxidative

stress, endothelial dysfunction, vascular inflammation, increased vascular reactivity and structural remodeling. Together, these responses lead to increased peripheral resistance and therefore to increased blood pressure[65, 66]. It is known that ROS modulate specific cellular pathways (redox signaling), leading to changes in gene transcription and in functional oxidative modifications of cellular proteins that cause cellular dysfunction[56,67,68]. Thus, oxidative stress not only causes direct and irreversible oxidative damage to macromolecules, but it also affects redox-dependent signaling in the vasculature[69]. ROS generation by ethanol is important to its pathophysiology in the cardiovascular system, as ethanol is extensively metabolized into acetaldehyde in the liver, mainly by the enzyme alcohol dehydrogenase[70]. Acetaldehyde, in turn, is oxidized to acetate by acetaldehyde dehydrogenase, which results in the generation of ROS and decreased NO levels[71].

In addition to the ROS generated during ethanol metabolism, some studies have shown the involvement and contribution of the nicotinamide adenine dinucleotide phosphate [NAD(P)H] oxidases to dysfunctions promoted by chronic ethanol consumption in several tissues[72-76]. Increased vascular oxidative stress induced by ethanol consumption is related to the activation of the enzyme NAD(P)H oxidase, and this mechanism is involved in the increased blood pressure caused by chronic ethanol consumption. NAD(P)H oxidase is the main source of ROS in endothelial and smooth muscle vascular cells[65], and it is considered a key factor in the vascular dysfunctions induced by ethanol. Husain *et al*[77] demonstrated that chronic ethanol consumption leads to an increased NAD(P)H oxidase activity and ROS generation that leads to membrane lipid peroxidation. The authors also observed increased phenylephrine-induced contraction and reduced acetylcholine-induced relaxation in aortas from ethanol-treated rats[77]. These data suggest that the initial step in the cardiovascular dysfunction associated with chronic ethanol consumption involves the formation of ROS, and this process can be mediated by the enzyme NAD(P)H oxidase. Moreover, this enzyme has been implicated in the activation of xanthine oxidase and the uncoupling of eNOS, which leads to ROS overproduction[78].

The antioxidant enzymes are the first line of defense against ROS-induced oxidative tissue injury. In vascular tissue, the enzymatic antioxidant system mainly consists of superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPx), thioredoxins and peroxiredoxins. The non-enzymatic antioxidants include ascorbate, tocopherol, glutathione, bilirubin and uric acid[79,80]. The antioxidant mechanisms antagonizing the consequences of chronic ethanol consumption have particularities related mainly to the type of tissue studied, the duration of treatment and the concentration of ethanol used. Das and Vasudevan[81] showed that ethanol consumption increased SOD activity and decreased CAT activity in a time- and dose-dependent manner[81]. Husain *et al*[82] demonstrated increased SOD activity in the liver of rats treated with ethanol[82]. It is known that SOD activity is modulated by increased ROS generation and by lipid peroxidation[83,84]. In rats, chronic ethanol treatment led to increased CAT activity and impaired the maintenance of the glutathione redox cycle in renal tissue, with an increase in GPx activity and a decrease in GSH (reduced glutathione) levels[84].

In clinical studies, increased plasma activity of SOD and GPx was observed in subjects who regularly consume ethanol[85,86]. Husain *et al*[87] demonstrated that chronic ethanol consumption by rats significantly depressed both cytosolic CuZn-SOD and mitochondrial Mn-SOD activities in the plasma, indicating an inability of the cells to scavenge superoxide anion. Moreover, plasma CAT and GPx activities were also significantly decreased in ethanol-treated rats. The inhibition of these enzymes may increase superoxide anion availability, which can react with NO to form peroxynitrite[87].

The role of oxidative stress in ethanol-induced hypertension is complex and may involve increases in ROS generation or reductions in antioxidant systems. The increase in oxidative stress promoted by ethanol is associated with endothelial dysfunction, vascular inflammation and increased vascular reactivity. These processes may contribute directly or indirectly to increased peripheral resistance and therefore to increased blood pressure.

NO bioavailability

In 1980, Furchgott *et al*[88], in classic study, discovered that endothelial cells produce an endothelium-derived relaxing factor (EDRF) in response to stimulation by acetylcholine. In 1987, Palmer *et al*[89] and Ignarro *et al*[90] identified EDRF as NO, a free radical that diffuses to underlying smooth muscle to induce vasodilatation[89,90]. These findings marked the beginning of a major worldwide expansion of research into the role of NO in vascular physiology and pathophysiology.

The endothelium plays a pivotal role as a sensor, transducer, and integrator of signaling processes regulating vascular homeostasis, and it is known that vascular diseases, including hypertension, are characterized by impaired endothelium-derived NO bioactivity. The effect of ethanol on the function of the endothelial is complex[91]. Appreciating the importance of NO in the maintenance of vascular tone, some studies have examined the mechanisms underlying the impairment of NO-mediated vasodilatation by chronic ethanol consumption[92]. In theory, such a decrease in NO bioactivity could result from reduced NO production or from the inactivation of NO[93]. NO is produced by NOS (nitric oxide synthase) *via* one of three isoforms: the neuronal NOS (nNOS/NOS1), inducible NOS (iNOS/NOS2), and the endothelial NOS (eNOS/NOS3)[94]. Ethanol exerts different effects on these isoforms in a variety of cells and tissues. Tirapelli *et al*[52] demonstrated that chronic ethanol consumption reduced the vascular expression of eNOS in female rats. Conversely, iNOS expression in arteries from ethanol-treated rats was significantly increased compared with control tissues. This response could be the result of a compensatory mechanism, where increased iNOS expression could induce a substantial and sustained release of NO

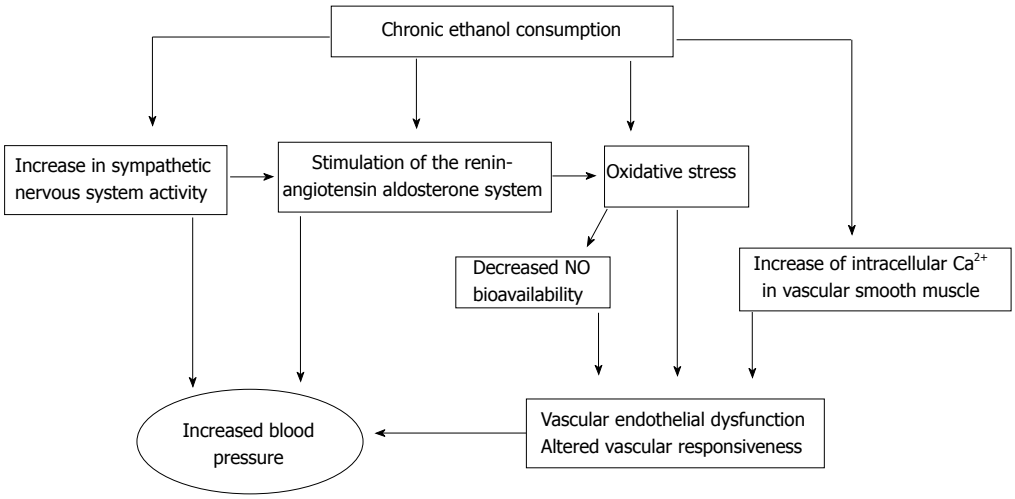

Figure 1 Summary of the basic pathophysiological mechanisms underlying ethanol-induced hypertension.

to compensate for the reduction of eNOS expression[52]. In the rat liver, ethanol decreased eNOS expression and activity[95]. Krecsmarik *et al*[96] demonstrated that chronic ethanol consumption induced an increase in iNOS activity and a decrease in nNOS expression in the rat gastrointestinal tract[96]. Moreover, chronic ethanol treatment reduced the eNOS-dependent relaxation of cerebral arterioles in rats[97].

The effect of ethanol on endothelial NO bioavailability appears to be related to the dose of ethanol. In this sense, it was shown that low concentrations of ethanol induced an increased release of endothelial NO due to the activation and expression of NOS[98,99]. Utkan *et al*[28] described that chronic ethanol consumption potentiates endothelium-dependent relaxation in aortic rings, most likely through interference with the synthesis and/or release of NO or adaptive alterations in muscarinic receptors on the endothelial cells[28].

While low concentrations of ethanol are described to increase endothelial NO production, the chronic consumption of high doses of ethanol impairs endothelial function in association with reduced NO bioavailability. Husain *et al*[44,100] described down-regulation of the NO-generating system, leading to impaired vasorelaxation and hypertension. Male Fisher rats orally administered 20% ethanol (4 g/kg - 12 wk) showed increased systolic and diastolic blood pressures and impaired vascular relaxation compared with controls. The expression of eNOS in the thoracic aorta isolated from ethanol-fed rats was down-regulated, leading to a depletion of aortic NO. This process may alter resistance vessel architecture, reducing its dilatory capacity[44,100]. In 2004, Kuhlmann *et al*[101] reported that high concentrations of ethanol decreased NO synthesis in and proliferation of endothelial cells from human umbilical veins.

The concentration of plasma asymmetric dimethylarginine (ADMA) in alcoholics is higher than in non-alcoholic subjects[102]. ADMA is an endogenous inhibitor of NO production, which is generated from the methylation of arginine residues by arginine methyltransferases and subsequent proteolysis. In this sense, increased ADMA levels could also contribute to the reduced bioavailability of NO in alcoholics.

NO, which is constantly formed, readily reacts with reactive molecules, such as superoxide anion[103,104]. Most of the cytotoxicity attributed to NO is due to peroxynitrite, which is produced from the reaction between NO and superoxide anion[105]. This loss of NO that occurs in the reaction with superoxide anion deprives vascular smooth muscle cells of NO. Ethanol reduces the bioavailability of NO through both the inhibition of eNOS and through the formation of peroxynitrite, which can lead to cellular damage[106].

CONCLUSION

The link between hypertension and chronic ethanol consumption is well established, and the mechanism by which ethanol increases blood pressure is complex. There appears to be more evidence implicating the sympathetic nervous system, the renin-angiotensin-aldosterone system, increased intracellular Ca^{2+} in vascular smooth muscle, oxidative stress, decreased NO bioavailability and endothelial dysfunction than there is evidence for the other mechanisms suggested, but this issue remains an open one. After a century of study, it is established that chronic ethanol consumption leads to hypertension and that this process is a multi-mediated event involving the aforementioned mechanisms (Figure 1). Thus, it is of great importance to invest in implementing strategies that help to prevent alcoholism, thus reducing the risk of ethanol-associated cardiovascular diseases.

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