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term ethanol consumption in rats. Because aging is known to be associated with clearly reduced arterial dilatation (7, 8), senescent rats were also included in the study. The present findings suggest for the first time that especially the potassium channel-related component of arterial relaxation could be augmented by chronic ethanol exposure.

METHODS

Animals and experimental design. Young (n = 27) and aged (n = 22) male Wistar rats were housed one to a cage in a standard experimental animal laboratory (illuminated 0800 to 2100, temperature +22°C) and provided standard chow (Altromin 1314, Petersen, Ringsted, Denmark) and drinking fluid (tap water) ad libitum. At the age of 3 and 29 mo, the young and old Wistar rats, respectively, were allocated to six groups (n = 7–10): control-young, sucrose-young, ethanol-young, control-aged, sucrose-aged, and ethanol-aged. Ethanol-fed rats were given 25% ethanol by gastric gavage three times a day (8 AM, 2 PM, and 8 PM) 4 days a week. Immediately before each ethanol feeding, the severity of ethanol-induced symptoms of each rat was evaluated by the use of the following 10-point symptomatized, six-level symptom scale (see Ref. 26): (0) neutrality, no signs of symptoms; (1) sedation, reduced muscle tone and motor activity, no impairment of gait or coordination; (2) walking is slightly impaired, but the rat is able to elevate the abdomen and pelvis; (3) clearly impaired walking, impaired elevation of abdomen and pelvis; (4) slowed righting reflex, no elevation of abdomen and pelvis; (5) loss of righting reflex, response to pain stimuli; (6) general anesthesia/coma, no response to pain stimuli but spontaneous breathing. The dose of ethanol was individually adjusted (gavaged volume in average 4–5 ml in different groups) according to the level of ethanol-induced symptoms as follows: 0 = 4.0–4.5 g/kg, 1 = 3.5 g/kg, 2 = 3.0 g/kg, 3 = 2.5 g/kg, 4 = 2.0 g/kg, 5 = 1.0 g/kg, 6 = 0 g/kg. The animals were kept at the levels 2–3 of the above symptom scale.

In the sucrose-young and sucrose-aged groups, the caloric content of the diet was adjusted to match that of the ethanol-exposed groups by administering sucrose by gastric gavage in a similar manner as ethanol. The sucrose-fed ethanol-exposed groups by administering sucrose by gastric gavage in a similar manner as ethanol. The sucrose-fed ethanol-exposed groups by administering sucrose by gastric gavage in a similar manner as ethanol.

After exposure 5 wk. Thereafter, the ethanol and sucrose feedings were withdrawn 62 h before the rats were decapitated and exsanguinated, and the superior mesenteric arteries were carefully excised and cleaned of adherent connective tissue. The experimental design of the study was approved by the Animal Experimentation Committee of the University of Tampere, Finland. Experimental animal data are given in Table 1.

Mesenteric arterial responses in vitro. Two successive endothelium-intact standard sections (3 mm in length) of the mesenteric artery from each animal were cut, beginning 5 mm distally from the mesenteric artery-aorta junction. The rings were placed between stainless steel hooks (diameter 0.3 mm) and suspended in an organ bath chamber (volume 20 ml) in physiological salt solution (PSS) (pH 7.4) of the following composition (mM): 119.0 NaCl, 25.0 NaHCO3, 1.1 glucose, 1.6 CaCl2, 4.7 KCl, 1.2 KH2PO4, 1.2 MgSO4, and aerated with 95% O2-5% CO2. The rings were initially equilibrated for 30 min at 37°C with a resting tension of 1.5 g. The force of contraction was measured with an isometric force displacement transducer and registered on a polygraph (FT03 transducer and model 7E Polygraph; Grass Instrument, Quincy, MA). The presence of intact endothelium in vascular preparations was confirmed by clear relaxation responses to acetylcholine (ACH, 1 µM) in rings that were precontracted with norepinephrine (NE, 1 µM).

Endothelium-dependent relaxations and receptor-mediated contractions. After the equilibration period, vascular responses to ACh were examined. The rings were precontracted with NE (1 µM). After the contraction had fully developed, increasing concentrations of the relaxing agent were cumulatively added to the organ bath. The next concentration of the agonist was added only when the previous level of the response was stable. After the maximal response had been reached, rings were rinsed with PSS and allowed a 20-min recovery period at resting tension. Responses to ACH were then elicited in the presence of cyclooxygenase inhibitor diclofenac (3 µM), after which NO synthase inhibitor Nω-nitro-L-arginine methyl ester (L-NAME, 0.1 mM) was also added to the bath and responses to ACH were retested. After the equilibration period, concentration-response curves for NE in the presence of diclofenac and L-NAME were cumulatively determined.

Relaxations to isoproterenol, nitroprusside, and cromakalim. After the equilibration period, cumulative relaxations to the β-adrenoceptor agonist isoproterenol, NO donor sodium nitroprusside, and K+ channel opener cromakalim were examined in rings precontracted with 1 µM NE, with a 20-min recovery period at resting tension between the responses. The maximal contractions to NE were presented in grams. The EC50 values for NE were calculated with a computer program and presented as the negative log of the (pD2), which values were also used in the statistical analysis. The relaxations to ACH, nitroprusside, isoproterenol, and cromakalim were presented as a percentage of the preexisting contraction force.

Compounds. The following drugs were used: acetylcholine chloride, dl-cromakalim, dl-isoproterenol hydrochloride, L-NAME hydrochloride, (Sigma Chemical, St. Louis, MO), diclofenac (Voltaren injection solution; Ciba-Geigy, Basel, Switzerland), L-norepinephrine-d-hydrogenatrate (Fluka Chemie, Buchs, Switzerland), and sodium nitroprusside (Merck, Darmstadt, Germany). The stock solutions of the compounds used in the in vitro studies were dissolved in distilled water. All solutions were freshly prepared before use and protected from light.
Analysis of results. Statistical analysis was carried out by ANOVA supported by Bonferroni confidence intervals in the case of pairwise between-group comparisons. When the data consisted of repeated observations at successive time points, ANOVA for repeated measurements was applied. Differences were considered significant when $P < 0.05$. The results were expressed as means ± SD or ± SE. The data were analyzed with BMDP statistical software.

RESULTS

Mesenteric arterial responses in vitro. Arterial relaxation of NE-precontracted rings to nitroprusside and cromakalim, agents that mediate arterial relaxation via the formation of exogenous NO and the opening of ATP-sensitive K$^+$ channels ($K_{ATP}$), respectively, did not significantly differ between young and aged rats. However, the relaxations to the $\beta$-adrenoceptor agonist isoproterenol were markedly impaired in aged rats when compared with those of the young control group (Fig. 1, Table 2). Responses to nitroprusside were not affected by the ethanol or sucrose treatments. Interestingly, ethanol intake clearly improved the vasorelaxations elicited by isoproterenol and cromakalim in both young and aged groups (Fig. 2, Table 2).

Fig. 1. Relaxations to nitroprusside (top), isoproterenol (top), cromakalim (middle), and acetylcholine (middle and bottom) in norepinephrine (1 µM)-precontracted, isolated endothelium-intact mesenteric arterial rings from young control rats and aged control rats. Relaxations to acetylcholine were induced in the absence and presence of 3 µM diclofenac and in the presence of diclofenac and 0.1 mM N$^G$-nitro-L-arginine methyl ester (L-NAME). Symbols indicate means ± SE; $n = 7$–10 rats in each group; $^* P < 0.05$, ANOVA for repeated measurements.
DISCUSSION

The major findings of the present work were the following: 1) arterial relaxations to the β-adrenoceptor agonist isoproterenol and the K\textsubscript{ATP} channel opener cromakalim were clearly augmented by ethanol consumption in both young and aged rats; 2) arterial relaxation responses to the endothelium-dependent agonist ACh and the NO donor nitroprusside were not affected by ethanol exposure; 3) in the young controls and in both ethanol-exposed groups distinct diclofenac- and L-NAME-resistant relaxations to higher concentrations of ACh were present, whereas only a minute relaxation was observed in the aged control group; and 4) sucrose feeding did not have a noticeable influence on arterial dilatation, suggesting that the caloric content of the ethanol diet did not play a significant role in the present findings. Taken together, these results suggest for the first time that especially the potassium channel-related component of arterial relaxation properties could be augmented by chronic ethanol exposure.

Arterial relaxations to the β-adrenoceptor agonist isoproterenol were reduced in aged rats when compared with young rat controls, which is in agreement with previous findings in humans and rats (6). Interestingly, these responses were clearly augmented by ethanol consumption in both young and aged rats. Vasodilatation to isoproterenol is predominantly endothelium independent via the stimulation of β-adrenoceptors and the subsequent increase in cAMP in smooth muscle (1). However, isoproterenol also hyperpolarizes blood vessels via K\textsubscript{ATP} and Ca\textsuperscript{2+}-activated K\textsuperscript{+} channels in the smooth muscle (25, 28). The present results whereby the responses to cromakalim, an opener of K\textsubscript{ATP}, were markedly improved in both young and aged ethanol-treated groups suggest that arterial relaxation via potassium channels was augmented following long-term ethanol intake. Therefore, enhanced K\textsuperscript{+} channel-mediated vasodilatation could also explain the improved relaxation to isoproterenol in both ethanol-exposed groups in this study. Because the mechanisms of action of isoproterenol and cromakalim are mainly endothelium independent, it is probable that favorable

Table 2. Parameters of contractile and relaxation responses of isolated endothelium-intact arterial rings

<table>
<thead>
<tr>
<th>Variable</th>
<th>Control</th>
<th>Sucrose</th>
<th>Ethanol</th>
<th>Control</th>
<th>Sucrose</th>
<th>Ethanol</th>
</tr>
</thead>
<tbody>
<tr>
<td>Contractions to norepinephrine pD\textsubscript{50}</td>
<td>6.96 ± 0.13</td>
<td>6.93 ± 0.11</td>
<td>6.59 ± 0.16</td>
<td>7.00 ± 0.21</td>
<td>7.07 ± 0.08</td>
<td>6.80 ± 0.19</td>
</tr>
<tr>
<td>Maximal force, g</td>
<td>2.9 ± 0.3</td>
<td>3.7 ± 0.2*</td>
<td>2.7 ± 0.4†</td>
<td>2.2 ± 0.3</td>
<td>2.7 ± 0.2</td>
<td>2.2 ± 0.2§</td>
</tr>
<tr>
<td>Relaxation responses pD\textsubscript{50}</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Acetylcholine</td>
<td>NC</td>
<td>NC</td>
<td>NC</td>
<td>7.29 ± 0.24</td>
<td>6.67 ± 0.15†</td>
<td>7.65 ± 0.13§</td>
</tr>
<tr>
<td>Isoproterenol</td>
<td>NC</td>
<td>NC</td>
<td>4.76 ± 0.13</td>
<td>5.43 ± 0.32</td>
<td>4.64 ± 0.17</td>
<td>5.86 ± 0.15§</td>
</tr>
<tr>
<td>Nitroprusside</td>
<td>7.01 ± 0.08</td>
<td>7.02 ± 0.10</td>
<td>7.01 ± 0.09</td>
<td>7.27 ± 0.10</td>
<td>7.22 ± 0.17</td>
<td>7.74 ± 0.17</td>
</tr>
<tr>
<td>Cromakalim</td>
<td>6.54 ± 0.09</td>
<td>6.39 ± 0.04</td>
<td>7.12 ± 0.06‡</td>
<td>6.43 ± 0.07</td>
<td>6.60 ± 0.03</td>
<td>7.09 ± 0.08§</td>
</tr>
<tr>
<td>Maximal relaxation (% of 1 μM norepinephrine-induced precontraction)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Acetylcholine</td>
<td>20.0 ± 5.0</td>
<td>21.8 ± 4.2</td>
<td>24.9 ± 5.1</td>
<td>78.5 ± 5.4*</td>
<td>69.7 ± 3.8</td>
<td>87.8 ± 3.2</td>
</tr>
<tr>
<td>Isoproterenol</td>
<td>46.0 ± 7.9</td>
<td>39.3 ± 6.7</td>
<td>83.1 ± 5.1*‡</td>
<td>67.7 ± 9.2*</td>
<td>72.6 ± 5.4</td>
<td>88.4 ± 3.8§</td>
</tr>
<tr>
<td>Nitroprusside</td>
<td>89.3 ± 2.3</td>
<td>87.3 ± 2.7</td>
<td>87.2 ± 1.8</td>
<td>95.3 ± 1.1*</td>
<td>95.3 ± 0.5</td>
<td>94.7 ± 1.2</td>
</tr>
<tr>
<td>Cromakalim</td>
<td>85.0 ± 4.3</td>
<td>87.6 ± 2.1</td>
<td>98.8 ± 3.0‡</td>
<td>82.4 ± 2.1</td>
<td>90.3 ± 1.2</td>
<td>94.5 ± 1.5‡</td>
</tr>
</tbody>
</table>

Values are means ± SE; n = 7–10 rats in each group. E\textsubscript{C50} values are presented at the negative logarithm (pD\textsubscript{50}) of concentration of the agonist. pD\textsubscript{50} values for acetylcholine and isoproterenol were not calculated in aged groups because maximal relaxations to these agonists did not reach 50% in the present study. NC, not calculated. *P < 0.05 compared with control-aged group; †P < 0.05 compared with control-young group; §P < 0.05 compared with sucrose-aged group (Bonferroni test).
influences of ethanol on these responses were mainly mediated via smooth muscle. However, because K⁺ channels are also expressed in endothelial cells, the actions of isoproterenol or cromakalim may partially have been mediated via the endothelium. Future investigations will clarify whether the influences of chronic ethanol consumption on the arterial relaxations to isoproterenol are mediated via effects on smooth muscle, endothelium, or both.

Arterial relaxations to nitroprusside were comparable in all study groups, indicating similar vascular sensitivity to NO in young and aged rats as well as the ethanol-exposed groups. Previously, the relaxation to nitroprusside has been suggested to remain unaffected or to be enhanced by aging in both humans and experimental animals (8, 20) and to remain unaltered by ethanol consumption in rats (14). The fact that the responses to nitroprusside were not affected by ethanol consumption in the present study suggests that improvement of general vascular relaxation properties (e.g., regulation of intracellular calcium) did not play a role in the enhanced isoproterenol- and cromakalim-
induced relaxations in the young and aged ethanol-fed groups.

Previously, aging has been associated with impaired endothelium-dependent dilatation (7, 8), a finding that was confirmed in the present study. The reports concerning the influences of ethanol on endothelium-dependent relaxations in experimental animals have been quite contradictory (11–14, 31). In the present investigation, chronic ethanol exposure had no significant effect on the endothelium-mediated relaxation induced by ACh (in the absence of inhibitors of cyclooxygenase and NO synthase) in both young and aged rats. Thus from the present findings it would appear that ethanol consumption does not considerably alter endothelial function in experimental animals.

Diclofenac improved the dilator response to ACh in all aged groups. This finding is in concert with the concept whereby endothelium-derived contractile factors (EDCF), the production of which depends on cyclooxygenase, were released from the endothelium of aged animals (16, 20, 21). EDCF have also been suggested to be involved in impaired endothelium-medi-
ated vasomotion in spontaneously hypertensive rats (18). However, the effect of diclofenac in the response to ACh did not significantly differ between the aged ethanol-treated and control groups, suggesting that the release of EDCF was not modified by ethanol exposure in the present study. Inhibition of NO synthase by L-NAME diminished the relaxations to ACh in all study groups. Because the endothelium-mediated response in the aged controls and sucrose-fed groups was nearly abolished by L-NAME, it was predominantly mediated via NO. However, all other groups showed distinct diclofenac- and L-NAME-resistant relaxations, suggesting that endothelial products other than NO were mediating the enhanced response to ACh. Recent investigations have indicated that endothelium-mediated relaxations that remain resistant to both NO synthase and cyclooxygenase inhibition are mediated by another vasoactive autacoid, the endothelium-derived hyperpolarizing factor (4). The chemical characteristics of endothelium-derived hyperpolarizing factor remain unknown, but functionally this factor is a K+ channel opener (4). Previously, endothelium-dependent hyperpolarization has been found to be impaired in aged rats (7), and the present findings support this view because the aged control rats only showed a minute relaxation to ACh in the presence of diclofenac and L-NAME. Because the relaxation responses induced by cromakalim, an opener of KATP, and the diclofenac- and L-NAME-resistant relaxations to ACh were improved by ethanol in young and aged rats, the present findings suggest that ethanol feeding enhanced arterial relaxation via potassium channel-mediated mechanisms. These findings suggest a novel mechanism of action of chronic ethanol exposure on the vasculature.

Chronic ethanol intake has previously been reported to induce desensitization to the vasoconstrictor action of phenylephrine in experimental animals (29). On the other hand, long-term ethanol consumption has been suggested to enhance vascular contractility to NE (15) and potentiate α-adrenergic contractions probably through an interference with the production or release of EDHF in experimental animals (24). However, in this investigation, arterial contractile sensitivity to NE was comparable in all study groups. Thus the present findings suggest that prolonged ethanol consumption does not considerably affect arterial contractile function in rats.

Because the aim of the present study was to evaluate the possible roles of chronic ethanol consumption on arterial function in young and aged rats, the animals were withdrawn from ethanol 2 days before the bioassay studies to completely eliminate the presence and influence of ethanol in vitro (3, 5, 9, 11–13) during the measurements. Thus theoretically the observed favorable influences on the control of arterial tone could have been due to the chronic ethanol consumption or to its withdrawal. However, withdrawal symptoms are known to disappear almost completely in rats in 58 h (26), and in the present study ethanol was withdrawn 62 h before vascular studies. Thus it seems unlikely that the withdrawal of ethanol would have such profound influences on arterial function that were observed in the present study.

In conclusion, arterial relaxation responses to cromakalim and isoproterenol, but not to ACh and nitroprusside, were clearly improved by ethanol exposure in young and aged rats. In addition, in the young controls and in both the young and aged ethanol-treated groups distinct diclofenac- and L-NAME-resistant relaxations to higher concentrations of ACh were present, whereas only a minute relaxation to ACh was observed in the aged control group under these conditions. Taken together, these results suggest that the potassium channel-related component of arterial relaxation could be augmented by ethanol feeding in rats.

This study was supported by the Aarne Koskela Foundation, the University of Tampere, the Medical Research Fund of Tampere University Hospital, and the Finnish Foundation for Alcohol Studies, Finland.

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Received 31 July 1998; accepted in final form 25 September 1998.

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