all these point to the fact that hornet venom acts similarly to anti-ChE agents. Such activities of the venom are reversible, because the injection of heparin (in addition to atropine) produces considerable improvement of the condition, probably freeing the receptors or the affected tissue from the venom, which probably has a higher affinity for heparin.


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## Ethanol reduces $Ca^{2+}$ concentrations in arterial and venous smooth muscle


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*Summary.* The present results, using isolated rat aortic strips and portal vein segments, demonstrate that ethanol (170–430 mM) significantly inhibits calcium uptake in these 2 different types of vascular smooth muscle.


Ethanol has been shown to inhibit spontaneous mechanical activity of isolated intestinal[2], uterine[3] and vascular smooth muscle[4]. Besides its direct action on blood vessels, ethanol was also demonstrated to attenuate arterial, arteriolar, venular and venous smooth muscle responsiveness to several vasoactive substances[4–6]. These direct and indirect effects of ethanol have been hypothesized to be brought about via its antagonistic or inhibitory effects on calcium ion flux in smooth muscle[2,4–6]. However, no direct evidence is, as yet, available for the latter tenet. The present study indicates that ethanol can, indeed, affect exchangeable and membrane-bound calcium in arterial and venous smooth muscle.

*Methods.* Thoracic aortas and portal veins, obtained from male Wistar rats (300–400 g), were cut helically and longitudinally, respectively, and set up isometrically, in vitro, as described previously[4]. The vascular tissues were equilibrated for 2 h in muscle chambers containing Krebs-Ringer bicarbonate solution (NKR), the composition of which has been reported previously[4]. Tissues bathed in NKR solution were aerated continuously with a 95% $O_2$–5% $CO_2$ mixture, and kept at 37 °C (pH 7.4–7.5). After the initial 2-h incubation period, the tissues were exposed to $^{45}Ca$ containing medium (0.004 μCi/ml) in the presence or absence of 170 and 430 mM ethanol for 30 min. These concentrations of ethanol were chosen since they markedly attenuate spontaneous mechanical activity and drug-induced contractions in aorta and portal vein[4–6]. At the end of 30 min, each tissue was rinsed in ice-cold NKR for 10 sec (conventional method) or for 2 or 5 min, respectively, in 50 mM $La^{+++}$, $Ca^{++}$-free medium (lanthanum method, as modified by Godfraind[7]), the composition of which is as follows (mmoles/l): NaCl, 118; KCl, 5.9; $MgSO_4$, 1.2; glucose, 10; Tris hydroxymethylaminomethane, 5.0; and $LaCl_3$, 50. The final pH of this solution was 7.15. The tissues were then blotted on ash-free filter paper, weighed and digested with the addition of 1 ml Nuclear Chicago Solubilizer (Amersham Searle Corp.) at 50 °C for 5 h. Following acidification and addition of scintillation fluid (POPOP, 6 g; PPO, 75 mg; Toluene, 1 l) $^{45}Ca$ was measured with a Searle Mark III Liquid Scintillation Counter. The counts were corrected for quenching and machine efficiency. The results were converted to $^{45}Ca$ tissue content according to the formula:

$$^{45}\text{Ca(mmole/kg wet wt)} = \frac{\text{cpm in muscle}}{\text{wet wt (kg)}} \times \frac{\text{mmole Ca/l medium}}{\text{cpm/l medium}}$$

Table 1. Influence of ethanol on $^{45}Ca$ uptake into rat aortic strips[a]

| Treatment | Total exchangeable calcium | 2-min $La^{+++}$-wash calcium fraction (membrane-bound) | 5-min $La^{+++}$-wash calcium fraction ($La^{+++}$-resistant) |
|---|---|---|---|
| Control | 4.22±0.11 (33)[b] | 2.27±0.10 (24) | 0.80±0.04 (12) |
| Ethanol | | | |
| 170 mM | 3.46±0.11 (6)[c] | 1.53±0.14 (6)[c] | 0.78±0.09 (6) |
| 430 mM | 3.76±0.13 (6)[c] | 1.58±0.10 (7)[c] | 0.74±0.06 (8) |

[a]Values are expressed in mmoles/kg wet wt. [b]Number of different animals examined. [c]Significantly different from control ($p<0.005$).

Table 2. Influence of ethanol on $^{45}Ca$ uptake into rat portal veins[a]

| Treatment | Total exchangeable calcium | 2-min $La^{+++}$-wash calcium fraction (membrane-bound) | 5-min $La^{+++}$-wash calcium fraction ($La^{+++}$-resistant) |
|---|---|---|---|
| Control | 3.07±0.18 (31)[b] | 2.17±0.08 (19) | 1.40±0.14 (10) |
| Ethanol | | | |
| 170 mM | 2.51±0.17 (5)[c] | 1.55±0.12 (6)[d] | 1.64±0.17 (5) |
| 430 mM | 2.19±0.06 (6)[d] | 1.50±0.09 (8)[d] | 1.35±0.12 (7) |

[a]Values are expressed in mmoles/kg wet wt. [b]Number of different animals examined. [c]Significantly different from control ($p<0.05$). [d]Significantly different from control ($p<0.001$).

The 'conventional method' (i.e., without $La^{+++}$ present) reveals the exchangeable total calcium of the tissues. With the lanthanum method, the $^{45}Ca$ content is proposed to represent exchangeable: a) membrane-bound calcium (2-min wash); and b) intracellular $La^{+++}$-resistant calcium (5-min wash), as the concentration of lanthanum used (50 mM/l) has been shown[7-9] to: a) replace calcium at superficial binding sites; and b) block influx and markedly retard efflux of calcium within the 5-min contact time. Means and SEM were calculated and compared for statistical significance by use of Student's t-test.

*Results.* Tables 1 and 2 summarize the results obtained in the presence and absence of 170 and 430 mM ethanol in aorta and portal vein, respectively. The data indicate that ethanol, at both concentrations, attenuates to the same degree the exchangeable total and membrane-bound calcium in aorta (table 1). On the other hand, in portal vein, although the same 2 pools of calcium were decreased by ethanol, total exchangeable calcium was lowered in a concentration-dependent manner (table 2). In both tissues, ethanol failed to exert effects on $La^{+++}$-resistant cellular calcium (tables 1 and 2).

*Discussion.* Previously, it was reported[4,5] that ethanol in these concentrations attenuated: a) $Ca^{++}$-dependent spontaneous mechanical activity; b) $Ca^{+++}$-induced contractile responses; and c) contractions induced by vasoactive agents, which are known to utilize different pools of calcium for their responses of rat aorta and portal vein (e.g. catecholamines, prostaglandins, angiotensin, vasopressin and KCl). Based on these observations, it was postulated that the direct, as well as the indirect, peripheral vasodilator effects of this alcohol are probably mediated through its influence on calcium ion movements or translocation. It is well-known that calcium ions play an important role in excitation-contraction coupling and in vasoconstrictor-vasodilator events of vascular smooth muscle and, thereby, regulate vasomotor tone[10,11]. Results obtained in the present study support the contention[4-6] that ethanol attenuates Ca- and drug-induced responses and spontaneous activity of arterial and venous smooth muscle by inhibiting calcium uptake. Since different vasoactive substances utilize different cellular sources of activator calcium ions (e.g., extracellular, membrane-bound or intracellular), the use of $La^{+++}$ in this study made it possible to examine the effects of ethanol on several different pools of calcium. Briefly, lanthanum is known to replace calcium ions at the membrane binding sites and inhibit its transport into and out of the cell[7,11,12]. Use of 50 mM $La^{+++}$ solution is known to promote calcium displacement during the first 5 min and thereafter markedly retard calcium efflux from intracellular stores in the tissues used here[7-9]. Ethanol significantly decreased membrane-bound calcium (2 min $La^{+++}$-wash fraction) while it did not affect $La^{+++}$-resistant (intracellular) calcium (5 min $La^{+++}$-wash fraction) in either aorta or portal vein (tables 1 and 2). Since this alcohol markedly decreases maximum contractile responses to vasoactive agents of large and microscopic blood vessels[4-6], it appears that: 1. the functional calcium pool is located either loosely or tightly bound on the membrane; and 2. ethanol exerts its vasodepressant (vasodilator) effects by its inhibitory action on these pools of calcium located on the membrane. It is of some interest to note here that ethanol has also been shown recently to inhibit calcium uptake in hepatic mitochondria[13] and cardiac sarcoplasmic reticulum[14]. In addition, our data demonstrate that ethanol can exert actions on arterial and venous calcium pools in a concentration (170 mM) known to be present in the blood of rats maintained solely under ethanol anesthesia[15]. Blood levels of ethanol approaching 100–170 mM always result in profound peripheral vasodilation[4].

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