TITLE: EFFECTS OF ETHANOL AND ACETATE ON MAC : THE ROLE OF ADENOSINE RECEPTORS.

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The mechanism by which acute administration of ethanol reduces the MAC of inhalational anesthetics is unclear. Possibilities include a direct action of ethanol or of its metabolites. Ethanol is metabolized in the liver to acetate.[1] Acetate is released into the circulation and is further metabolized in the CNS resulting in the production of adenosine.[1] Since adenosine causes sedation and reduces the MAC for halothane,[2] it might mediate the effects of ethanol and acetate on MAC. The purpose of this study was twofold: (1) to establish dose-response relationships for ethanol and acetate on the MAC of isoflurane (Iso), and (2) to determine the role of adenosine receptors in mediating the effects of ethanol and acetate on MAC.

With approval from the university animal care committee, fasted, male Sprague-Dawley rats weighing 220 -260 g were studied. MAC of Iso was determined using standard techniques. Throughout the experiments, temperature was monitored with a rectal probe and maintained with an overhead radiant heating lamp. The inspired concentration of Iso was monitored continuously using a calibrated infrared medical gas analyser.

The dose-response relationship between ethanol and the MAC of Iso was established at doses of 0 (saline control, n=8), 1.0 (n=8), 2.0 (n=8) or 3.0 (n=8) gm/kg ethanol given by intraperitoneal (ip) injection. After 20 minutes, the MAC of Iso was determined. Similarly, the effect of acetate on MAC was determined at doses of 0 (n=8), 1.0 (n=8), 2.0 (n=8) or 3.0 (n=8) gm/kg ip. The serum concentration of acetate was determined 1 hour after the injection of ethanol or acetate.

The role of adenosine receptors in mediating the effects of ethanol and acetate on MAC was investigated by pretreating 24 rats with the adenosine receptor antagonist 8-phenyltheophylline (8-PT)12 mg/kg ip, 20 minutes before the administration of ethanol 1gm/kg (n=8), acetate 1gm/kg (n=8) or an equal volume of saline ip (n=8). After an additional 20 minutes, the MAC of Iso was determined. Data are presented as mean values ± SEM. Statistical significance ($p < 0.05$) was determined using ANOVA.

We found that the MAC of Iso was 1.26 ± 0.05 %. Both ethanol and acetate produced a significant dose-related reduction in the MAC of Iso (fig 1). 8-PT did not significantly affect MAC when given alone and did not affect the MAC reducing property of ethanol. In contrast, 8-PT reversed the MAC reducing property of acetate by 85 % (fig 2). The serum concentrations of acetate were 0.20 ± 0.10, 1.19 ± 0.12, and 1.52 ± 0.31 mmol/L in rats given saline, ethanol or acetate respectively.

We conclude that ethanol reduces MAC through a mechanism that does not involve adenosine receptors. In contrast, acetate, an important intermediate of cellular metabolism, produces a dose-dependent reduction in the MAC of Iso which is mediated mainly by adenosine receptors. This new finding suggests that acetate, which was previously thought to be devoid of CNS activity, has depressant effects on the CNS that are distinct from those of ethanol.

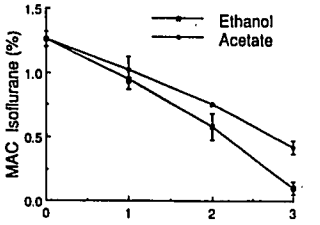

Figure 1

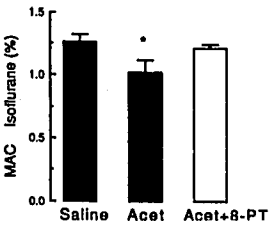

Figure 2 (* $p < 0.05$ vs controls)

References: 1. J. Clin. Invest. 41:9555-61; 1962
2. Life Sciences 42:1355-60; 1988

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Title: EVIDENCE FOR THE EXISTENCE OF INOSITOL PENTAKIS-/HEXAKISPHOSPHATE IN THE HEART BY A NEW PICOMOLAR-MASS HPLC ANALYSIS

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Stimulation of cell-surface receptors initiates hydrolysis of membrane-bound inositol lipid, which produces at least two second messengers inositol 1,4,5-trisphosphate (1,4,5-IP_3) and diacylglycerol. 1,4,5-IP_3 has been shown to mobilize intracellular calcium in several tissues. It can be phosphorylated to inositol 1,3,4,5-tetrakisphosphate (1,3,4,5-IP_4) which has been assumed to initiate calcium entry from the exterior (1). We have recently shown that in the heart 1,4,5-IP_3 may initiate the positive inotropic effect of α_1-adrenoceptor agonists and 1,3,4,5-IP_4 maintains the increase in force of contraction (2).

Almost all data published are based on radioisotopic techniques, which allow only relative estimates of fluxes. Absolute concentration data are still missing and there are a lot of questions about the role of inositol phosphates in vivo. In this study a recently published non-radiometric HPLC technique (3) was used. The experiments were performed on isolated spontaneously beating rat hearts at a Langendorff perfusion apparatus. Force of contraction was recorded. The hearts were preincubated with propranolol (1 μM) to avoid any interference from ß-adrenoceptor activation and incubated with the α_1-adrenoceptoragonist phenylephrine (0.01-100 μM). Thereafter the hearts were frozen, homogenized, charcoal treated and a solid-phase extraction was performed. Samples were freeze-dried, dissolved and on-line analyzed by HPLC.

The phenylephrine-induced positive inotropic effect was concentration-dependent and reached a maximum at about 5 min and remained constant thereafter. With the HPLC technique used in this study we could demonstrate an unknown IP_3 and IP_4 isomer selectivity in the heart. In addition, we described for the first time the existence of inositol pentakisphosphate (IP_5) and hexakisphosphate (IP_6) in the heart. We have also measured absolute concentration changes in these various inositol phosphates after α_1-adrenoceptor stimulation. Thus, the question is whether these novel inositol phosphates may function as second messengers too, although the metabolic pathways responsible for generating these inositol phosphates are not established. However, with this HPLC technique it is possible to measure inositol phosphates directly and quantitatively in various tissues and to investigate the role of this signalling system, which regulates intracellular calcium, for anaesthetic and drug actions in vivo.

References: (1) Berridge MJ, Irvine RF, Nature 341:197-205, 1989; (2) Kohl C, Schmitz W, Scholz H, Scholz J, Circ Res 66:580-583, 1990; (3) Mayr GW, Biochem J 254:585-591, 1988