Title: HALOTHANE CAUSES CORONARY VASODILATION IN THE ISOLATED ARRESTED PERFUSED RAT HEART

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INTRODUCTION: The coronary arterial microvasculature adjusts flow in order to match coronary O_2 supply with demand. The autoregulatory link between microvascular tone and myocardial metabolism makes it difficult to separate halothane's *direct* actions on the coronary vasculature from its *indirect* vascular effects resulting from lowered myocardial O_2 demand and altered intramural forces. Because of controversy over the ability of halothane (H) to cause direct coronary vasodilation,[1] we examined the effect of H on coronary vascular resistance (CVR) in an isolated arrested perfused heart model which eliminates the metabolic and compressive linkages between the myocardium and the coronary vasculature.

METHODS: We perfused hearts from male Sprague-Dawley rats (412±6g, $n=10$) by a modified Langendorff technique. Rats were killed instantly without anesthesia, in accordance with guidelines of our Institutional Animal Care and Use Committee. Prior to data collection, non-recirculating perfusion (60mmHg mean) was performed with a modified Krebs-Henseleit buffer equilibrated with O_2:CO_2 containing glucose 15 mM. The left ventricle was drained to atmospheric pressure through the apex. Coronary flow (CF) was measured by weighing the timed collection of buffer effluent from the heart. Aortic sufficiency was confirmed by the lack of systolic buffer ejection from the ventricular drain. Recirculating perfusion then was initiated at a mean perfusion pressure of 80 mmHg and a buffer volume of 15ml. Recirculating buffer was identical to the non-recirculating buffer except for the addition of bovine serum albumin, 0.1% and bovine insulin, 400 $\mu U \cdot ml^{-1}$. Cardiac arrest was produced by Na^+ channel blockade with tetrodotoxin (TTX), 18 $\mu g \cdot ml^{-1}$ buffer.

CF was measured in all hearts 3 times during the first 31 minutes of recirculating perfusion (equilibration). Hearts then were assigned to the *control (no H) group*, or the *halothane (H) group*. H (thymol-free) in O_2:CO_2 was delivered at 0.5% and 1.5% sequentially. [H] in outlet gas was confirmed by mass spectrometry. Equilibration of H with the buffer occurred by diffusion in the oxygenator circuit.

CF was measured 15 and 38 min after each [H] change in the H group, and at identical times in the no H group. Following the last CF at [H]=1.5%, anesthetic to the H group was discontinued. CF was measured in all hearts 15 and 38 min later to assess reversibility of the H effect. Finally, the potent vasodilator adenosine (10^{-5} or 10^{-6}M), was administered to all hearts and peak CF measured.

Because perfusion pressure was maintained constant, CF was inversely proportional to CVR. CF vs perfusion time in individual hearts was examined by regression analysis (excluding equilibration, reversibility, and adenosine data). Slopes from individual hearts were pooled by group, significance of changes in CF within and between groups were determined using paired and unpaired t-tests. $P<0.05$ was considered significant. Data are reported in $ml \cdot min^{-1} \cdot g\ dry^{-1}$ as mean ± SEM.

RESULTS: Contractile activity ceased with recirculating perfusion. There were no statistically significant differences between no H and H groups in body weight; wet, % dry, and dry heart weight; and initial CF. In the no H group, the slope of the regression of CF *vs.* perfusion time was not significantly different from zero ($P=0.091$). In the H group, the regression slope of CF *vs.* perfusion time was 0.083 ± 0.023 ($P=0.022$ vs 0). The latter value also was significantly different from the no H group slope ($P=0.016$).

H group hearts ([H]=0.5%) had a higher CF at the second measurement period than the corresponding no H hearts(103 ± 20 *vs.* 82 ± 10 respectively), these values were not statistically different ($P=0.38$). At [H]=1.5%, CF was significantly higher than in no H (136 ± 23 *vs.* 60 ± 10; $P=0.014$, 1-tailed t-test). CF in H group hearts rose significantly between 0.5 and 1.5% H (+33.1 ± 6.0; $P=0.0027$ 1-tailed paired t-test). In H group hearts, CF returned to baseline levels after discontinuing H ($P=0.24$). Adenosine elicited a similar CF in both groups (164 ± 22, no H *vs.* 150 ± 23, H group; $P=0.67$).

DISCUSSION: Intact animal studies have generally supported the hypothesis that H increases CVR secondary to its myodepressent activity while suggesting a mild component of *direct* vasodilation.[2,3] Other studies in intact animals have shown either decreased or no net change in CVR by H.[1] Isolated large coronary preparations, which circumvent some interpretational problems of whole animal data, indicate that H provokes vasodilation,[4] but do not examine the coronary microvasculature.

The isolated perfused rat heart is a metabolically and mechanically stable system which provides an isolated *micro*vascular bed where the *direct* effects of vasoactive substances can be studied under controlled conditions. TTX arrest of the heart should cause cardiac work and O_2 consumption to remain constant, unlinking anesthetic-induced effects and their secondary vascular autoregulatory actions from direct vascular effects.

Our data suggest: **1)** H causes a dose-dependent reduction in CVR, indicating a significant direct coronary vasodilatory effect of H, predominantly at the microcirculatory level; **2)** coronary vasodilation by H is reversible, demonstrating that vasoreactivity is conserved over the study period; **3)** the magnitude of vasodilation produced by [H]=1.5% is similar to that caused by the potent coronary vasodilator adenosine at near-maximal doses. It is difficult to postulate a further increase in CF at [H]>1.5%, this needs further study; **4)** H at clinically relevant concentrations may greatly reduce CF reserve in the rat; **5)** the TTX-arrested isolated perfused heart is a useful model for studying pharmacological mechanisms in the intact myocardial microcirculation.

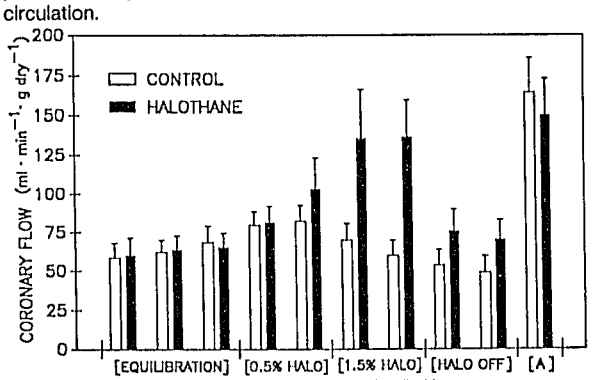

FIGURE: Changes in coronary flow due to halothane and adenosine in isolated arrested perfused rat hearts.

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