Title: HALOTHANE SELECTIVELY ATTENUATES α_2-ADRENOCEPTOR MEDIATED VASOCONSTRICTION IN A RAT HINDQUARTER MODEL

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Introduction

We developed a model of resistance vessel vasoconstriction isolated from cardiac and reflex effects to examine the purely vascular pharmacologic interactions between halothane and postjunctional α-adrenoceptor subtype mediated processes.

Methods

Hindquarter perfusion technique: A hindquarter perfusion model was developed for measuring anesthetic responses of a functionally isolated portion of the peripheral vasculature in pithed rats. Thirty three male Sprague Dawley rats weighing 350 to 500g were anesthetized with 2% inspired halothane in oxygen. A tracheotomy was performed and controlled ventilation instituted to maintain an end tidal CO_2 concentration ($ETCO_2$) between 4 to 6%. The rats were pithed to destroy the brain and spinal cord and the inspired gas mixture was changed to oxygen only.

A midline abdominal incision was made to expose the abdominal aorta. Following heparinization the aorta was cannulated proximally below the renal artery and distally above the iliac bifurcation with an external pump designed to deliver a constant rate of flow under variable load conditions. During perfusion the perfusion pressure (PP) to the rat hindquarter was measured via the distal aortic cannula. The abdominal aorta was completely interrupted by the external circuit.

Albumin (4%, 1 to 6ml) was given to produce a mean systemic arterial pressure (MSAP) of 30 to 40mmHg. Nonpulsatile hindquarter perfusion was initiated and pump flow rate was adjusted to produce a PP equal to the MSAP±5mmHg. Ventilation was adjusted and sodium bicarbonate administered as needed to maintain pH7.32 to 7.42, $PaCO_2$ 35 to 45, and PO_2 >100mmHg.

Halothane antivasoconstriction: After the animal had stabilized during hindquarter perfusion, vasoconstrictor drugs (agonists, see below) were given by continuous intra-arterial infusion through the distal aortic cannula at a constant rate titrated to produce an increase in perfusion pressure up to 100%. Vasoconstrictor drugs used were norepinephrine (NE, $\alpha_{1,2}$ agonist), phenylephrine (PE, α_1 agonist), and azepexole (AZP, α_2 agonist). When agonist-induced vasoconstriction had stabilized, halothane was administered to the rats lungs to produce end tidal halothane concentrations ($F_{H(ET)}$) of 0.125, 0.25, 0.5, 1.0, 1.5, and 2.0% in sequence. The halothane antivasoconstrictor dose response was determined for each agonist by calculating the fractional inhibition of agonist induced vasoconstriction at each $F_{H(ET)}$ as follows: PP inhibition (%)=$(PP_a-PP_h)\times100/(PP_a-PP_b)$ where PP_a=perfusion pressure during α-agonist infusion only, PP_h=perfusion pressure during α-agonist infusion and halothane inhalation, and PP_b=baseline perfusion pressure after stabilization to hindquarter perfusion (control). Regression analysis was used for individual rat data. The mean and SEM for slope and intercept data from these regressions were computed after pooling by α-agonist group. Differences between means were determined by Student's *t* test, and considered statistically significant at $p<0.05$.

Results

The doses of α-agonist required to increase PP were: NE 0.1-0.8$\mu g \cdot kg^{-1} \cdot min^{-1}$, PE 5-28$\mu g \cdot kg^{-1} \cdot min^{-1}$, and AZP 0.5-5$mg \cdot kg^{-1} \cdot min^{-1}$. Infusion of all α-adrenoceptor agonists produced dose dependant increases in both the MSAP and PP. The addition of halothane to inspired ventilatory gases produced a progressive decrease in MSAP for all rats.

The PP inhibition vs. $F_{H(ET)}$ data for each α-agonist are presented in figure 1 (mean±SE). Mean inhibition of AZP-induced PP was 46% ± 10 at $F_{H(ET)}$=2%. The slopes of the regression lines for NE and PE are 4.4 ± 4.8 and -1.3 ± 3.0 respectively, which are not significantly different from 0 nor from each other. The slope of the AZP regression line is 33 ± 5 which is significantly different from 0 ($p<0.0001$). A 30% inhibition of the AZP-induced increase in PP (ED_{30}) was produced by $F_{H(ET)}$=1.2%.

Discussion

To functionally isolate and evaluate halothane's effect on vasoconstriction mediated by postjunctional α-receptor mechanisms *in vivo*, we modified the rat hindquarter perfusion technique first described by Brody *et al*[1]. Perfusion of the hindquarter by constant flow perfusion isolates the vascular bed from cardiac effects which can alter blood pressure, so that PP can be used as an index of vascular resistance in the hindquarter. We also pithed our rats to: 1) to eliminate sympathetic nervous system activity which can produce a variable endogenous source of NE, 2) to eliminate halothane's influence on VSM tone through CNS sympathetic outflow, ganglionic neurotransmission, and prejunctional norepinephrine release, and 3) to alleviate the need for anesthesia during the control period.

Our results suggest that halothane attenuates α_2-adrenoceptor mediated vasoconstriction but does not significantly alter α_1-adrenoceptor mediated vasoconstriction in a rat hindquarter perfusion model of resistance vessel vasoconstriction. This conclusion is in agreement with data previously reported for pithed rats and isolated canine saphenous vein rings[2], and extends that previous work by examining rat resistance vessels directly. Further research is needed to define the intracellular mechanisms by which halothane affects the link between adrenoceptor activation and vascular smooth muscle contraction.

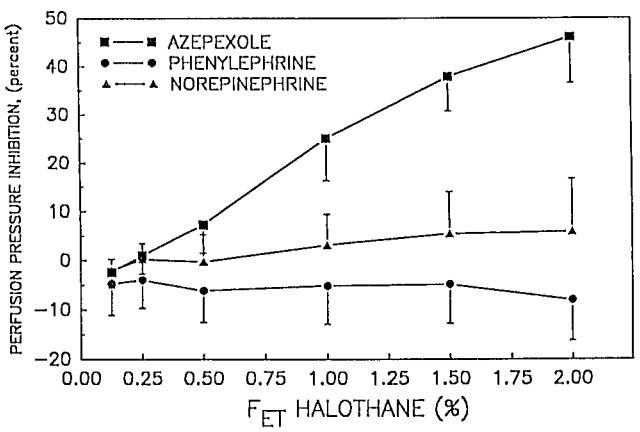

FIGURE 1. Maximal inhibition of hindquarter vasopressor responses at various $F_{H(ET)}$. Data are expressed as the mean ± SE.

References

1. Brody MJ, Shaffer RA, Dixon RL: A method for the study of peripheral vascular responses in the rat. J Appl Physiol 18:645-647,1963

2. Larach DR, Schuler HG, Derr JA, Larach MG, Hensley FA, Jr., Zelis R: Halothane selectively attenuates alpha-2 adrenoceptor mediated vasoconstriction, *in vivo* and *in vitro*. Anesthesiology 66:781-791, 1987