Title : HALOTHANE/DOBUTAMINE: MYOCARDIAL BETA-ADRENORECEPTORS

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Introduction. The positive inotropic action of catecholamines on the heart is associated with an increase in cyclic 3',5'-AMP (cAMP) content in the myocardial cell. It has been shown that dobutamine, a new congener of dopamine, has a positive inotropic action and increases the cAMP content of heart tissue, an effect thought to be mediated through beta-adrenergic receptors (1). Halothane has a negative inotropic action on the heart and has been shown to inhibit catecholamine-induced increase in myocardial adenylate cyclase (AC) activity (2). We have also found that halothane has no significant effect on the binding affinity to beta-adrenergic receptors of ^{3}H-dihydroalprenolol (^{3}H-DHA), a beta-antagonist, the number of receptors, and the ability of l-isoproterenol to displace bound ^{3}H-DHA in a canine myocardial membrane preparation. In this study we compared the binding affinity of dl-dobutamine to canine myocardial beta-adrenergic receptors, and its ability to activate AC, with that of l-isoproterenol, and the effect of halothane on these functions.

Methods. Myocardial membrane preparations were obtained from the pellet after centrifugation at 10,000 x g for 12 minutes. AC activity in this preparation was measured by the method of Krishna et al (3) and beta-adrenergic receptor binding, by displacement of ^{3}H-DHA (4). Halothane in air was delivered to the incubation mixtures via a manifold and its concentration was measured with a flame ionization detector. For AC assay, the mixture was equilibrated with the gas mixture for 20 minutes at 4 C before and during the 5 minute incubation at 37 C. For the adrenergic receptor binding determinations, the assay mixture was exposed to halothane for 30 minutes at 30 C. Control samples were exposed to breathing air by means of an identical manifold.

Results. The response of AC to the catecholamines in this partially purified membrane fraction was variable, presumably because GTP, a soluble membrane constituent thought to be essential for optimal hormonal response, is washed out during preparation. When GTP, 1×10^{-4}M, was added to the incubation mixture, the basal activity of AC was increased by 47% (from 114.4 ± 8.3 to 168.5 ± 10.9 pmol/mg protein/5 minutes). Under these conditions, dl-dobutamine, $2.5 \times 10^{-6} - 10^{-5}$M, stimulated AC in a concentration-dependent fashion with a maximal increase of 35% above control, while l-isoproterenol, 2.5×10^{-6}M, increased the activity by 115% above control. Halothane (5 vol%) did not significantly change the maximum response of AC to dobutamine and isoproterenol or to GTP.

dl-Dobutamine, like l-isoproterenol, displaced ^{3}H-DHA. However, its affinity for the receptor was 20-40 times lower than that of l-isoproterenol (figure). Halothane (5 vol%) did not alter the ability of dobutamine to displace ^{3}H-DHA from adrenergic receptors.

Discussion. The results demonstrate that dobutamine, like isoproterenol, binds to beta-adrenergic receptors and that this binding is associated with dose-dependent activation of AC, events typical for a beta-adrenergic agonist. When compared to l-isoproterenol, dl-dobutamine has about a 25-fold lower affinity for beta-adrenergic receptors and a lower intrinsic activity on the enzyme. As with isoproterenol, halothane has no effect on displacement of ^{3}H-DHA by dobutamine. The inability of halothane to depress catecholamine-stimulated AC activity in this membrane preparation is in contrast to results obtained with the whole homogenate and indicates that probably some component of the cell membrane essential for this effect of halothane may be altered or lost during the preparation of the membrane fraction. It may be that another soluble component is required for halothane depression of catecholamine-stimulated AC.

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References.

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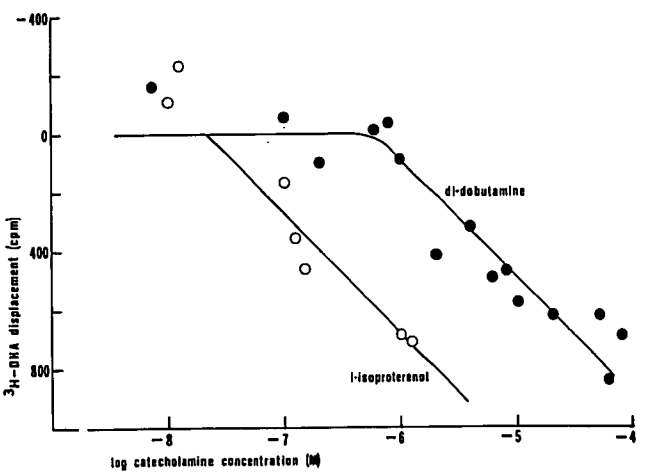

Displacement of ^{3}H-dihydroalprenolol by l-isoproterenol and dl-dobutamine

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