Introduction

Guanine nucleotide binding proteins (G proteins) couple receptors to a variety of effector systems. These proteins are heterotrimers, consisting of \( \alpha, \beta \) and \( \gamma \) subunits. Activation of G proteins by agonist-occupied receptors results in GTP-GDP exchange at the \( \alpha \)-subunits, followed by dissociation of the \( \beta\gamma \) subunits. In the case of \( G_s \), the \( \alpha_s \)-GTP species can then activate adenylate cyclase directly. Inhibition of adenylate cyclase, on the other hand, results from the binding of \( \beta\gamma \) released from the dissociation of \( G_i \) with free \( \alpha_s \)-GTP, thereby preventing activation of adenylate cyclase. Termination of G protein activation is mediated by GTPase activity intrinsic to the \( \alpha \) subunits, which cleaves \( \alpha \)-GTP to inactive \( \alpha \)-GDP. The resulting \( \alpha \)-GDP complexes can then reassociate with the \( \beta\gamma \) subunits to reform the heterotrimeric proteins (Gilman, 1987).

The normal activity of the \( G_i \) protein can be disrupted by several agents. For example, pertussis toxin uncouples receptors from \( G_i \) by catalyzing the mono-ADP-ribosylation and inactivation of the \( \alpha_i \) subunits. In addition, phorbol esters (Bell et al., 1986) and divalent cations such as \( \text{Mg}^{2+} \) and \( \text{Mn}^{2+} \) (Katada et al., 1985) can also inhibit the activity of \( G_i \). In this report, we present data showing that several of the newer positive inotropic drugs with methylxanthine-like ring structures can also inhibit the function of \( G_i \) and \( G_s \) (to a lesser extent) by interfering with GTP-GDP exchange.

The newer positive inotropic drugs, such as sulmazole, increase cAMP levels in cardiac cells. This is believed to be mediated by inhibition of a low \( K_m \) cAMP phosphodiesterase activity (Kariya et al., 1982) characterized in cardiac tissue. However, despite substantial investigation,
the relationship between phosphodiesterase inhibition and cardiotonic efficacy remains unclear (Harrison et al., 1986). In addition, these agents are effective inhibitors of the $A_1$ adenosine receptors (Parsons et al., 1988a), thereby relieving tonic inhibition of adenylate cyclase by endogenous adenosine. Increases in cAMP accumulation correlate well with the inotropic and vasodilatory actions of these drugs (Endoh et al., 1982; 1985), although other mechanisms independent of cAMP might account for these actions (Endoh et al., 1986).

Methods

For in vivo ADP-ribosylation of $\alpha_i$, pertussis vaccine (~ 300 opacity units/kg; 0.3-0.5 ml) was administered intraperitoneally to rats three days prior to sacrifice, as described previously (Parsons et al., 1988a). All other methods are as described in the figure legends.

Results

The new cardiotonic agent, sulmazole, stimulated adenylate cyclase activity in a dose-dependent manner, with a greater than 2-fold increase in activity over papaverine at the highest concentration of the drug tested (figure 1A). At a concentration of 100 $\mu$M, papaverine fully inhibited the low $K_m$ cAMP phosphodiesterase ($IC_{50}$ for inhibition being $3.8 \pm 1.3 \mu$M). Furthermore, the addition of sulmazole to papaverine (100 $\mu$M) neither increased nor decreased the maximal inhibitory effect of papaverine on phosphodiesterase activity. Therefore, the additional stimulatory effect of sulmazole on adenylate cyclase must be independent of its inhibition of the low $K_m$ cAMP phosphodiesterase activity ($IC_{50}$ for inhibition being 150 $\mu$M). Interestingly, the stimulatory effect of sulmazole is negated by the $A_1$ adenosine receptor agonist, R-PIA (figure 1B).