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EFFECTS OF TRYPAN BLUE ON THE ACTION OF ADRENERGIC AGONISTS IN THE GUINEA-PIG ISOLATED ATRIUM

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Abstract : It has been reported that trypan blue, a diazo dye with polyamphipathic structure, can inhibit the coupling of receptors to G-proteins. The present study was carried out to investigate the effect of trypan blue on the actions of adrenoceptor agonists in the guinea-pig atrium. Trypan blue (10 and 100 μM) antagonized the positive inotropic effects of isoprenaline and dobutamine by shifting their concentration-response curves to the right. With the selective β_2–adrenoceptor agonist, salbutamol, there was a reduction of response in the presence of trypan blue. Therefore, we concluded that trypan blue diminish the response to β–adrenoceptor agonists possibly via decoupling receptors from Gs. Trypan blue and similar agents, due to their unique mode of action, can be used as tools for the investigation of the mechanism of receptor-G protein coupling in the whole tissue preparation.

Key words : dobutamine, guanine-nucleotide binding protein, guinea-pig atrium, isoprenaline, salbutamol, trypan blue

INTRODUCTION

G-protein coupled receptors (GPCRs) are a large family of receptors which share common structural futures such as having seven transmembrane domains connected by intra- and extracellular loops. They affect intracellular processes by interaction with G-proteins, which are heterotrimeric consisting of α, β, γ, subunits (1, 2). A number of biochemical (3–8) and modelling studies (9) have suggested that the third intracellular loop as well as the C-terminal of GPCRs may interact with G-proteins. Compounds, such as mastoparan, could directly activate G-proteins (10). This receptor- independent mode of action was also observed with cationic amphiphillic neuropeptides and venom peptides (10). By contrast there are several compounds (i.e., heparin, suramin, and trypan blue) which could uncouple receptors from G-proteins and inhibit ligand induced response (3, 11). Trypan blue is a tetrasulfone diazo dye that readily enters into the cells, but it is only trapped in died cells (12–16). It has an amphipathic characteristic (3). Compounds that have such a characteristic including

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trypan blue analogs, heparin and suramin can reduce the affinity of α_2 and β_2 adrenoceptor to their agonists but not to antagonists (3). These compounds do not interact at the ligand binding site of the receptor or at the GTP binding site of the G–proteins (3). Therefore, it has been suggested that they can produce their effect by uncoupling the receptors from G–proteins (3). Also Dasso and Taylor have reported that these compounds could uncouple α_1–adrenoceptors from G–proteins (11). In whole tissue, the effects of trypan blue analogs are more complicated. It has been reported that they could antagonize P_2x-purinoceptors effects in vas deferens, (17, 18) as well as salbutamol relaxation effect in guinea-pig ileum (19). Therefore, the present study was carried out to investigate the effect of trypan blue in the guinea-pig atrium, to provide information about the effects of adrenoceptor-G protein interaction in whole tissue preparation.

METHODS

Preparation : Female albino guinea-pigs (150–500 g) were given a sharp blow to the back of the head and their hearts were excised and transferred to a physiological solution (NaCl 8.0; KCl 0.2; $MgCl_2$ 0.1; $CaCl_2$ 0.2; NaH_2PO_4 0.05; $NaHCO_3$ 1.0; Glucose 1.0 g/L) which was continually gassed with a mixture of 95% O_2 and 5% CO_2. The left atrium was dissected and transferred to a 50 ml bath containing physiological solution and maintained at 37°C. An initial load of 0.5 g was applied to the preparation. Two elctrodes on a glass hook located near the tissue delivered electrical stimuli to the muscle by electrical impulses of 2.5 Hz, 5 ms, and 25V as described by Burnstock et al (20). The mechanical activity was measured isometrically by means of a force transducer and recorded on a Beckman polygraph. The preparations were allowed to equilibrate for 60 min before the administration of drugs. The bathing solution was changed every 15 min during the equilibrium period. In all experiments with trypan blue, this agent was added 5 min before the addition of other drugs. Cumulative concentrations of the agonists were used in the absence and presence of trypan blue (10 and 100 µM), in the same tissue. Following agonists were used: Salbutamol (1, 10 and 100 µM) isoprenaline (0.1, 1, 10, 50, 100 and 1000 *f* M) and dobutamine (0.01, 0.1, 1, 10 and 100 µM). Concentrations refer to the final concentration of drugs in the incubation medium.

Drugs : Trypan blue, salbutamol and isoprenaline obtained from Sigma Chemical Company, USA. Dobutamine solutions were prepared from Dobutrex(R) vials manufactured by Eli Lilly Indianapolis, USA.

Statistical : The statistical significance was evaluated using paired Student's t-test. EC_{50} was calculated by probit program, (Pharm/Pcs -version 4).

RESULTS

Effects of trypan blue *per se* on atrial contractions

Trypan blue alone has no significant effect on the electrically paced atria contractions.

Effects of trypan blue on the dobutamine contraction- response curve and EC_{50} value

Trypan blue of 10 µM concentration diminish positive inotropic effect of dobutamine by shifting its concentration-

response curve to the right and at 100 µM concentration it not only shifted the curve to the right but also depressed the maximal efficacy of dobutamine (Fig. 1). The EC_{50} values were reduced significantly in both treatments (Table I).

Effects of trypan blue on the isoprenaline concentration-response curve

Trypan blue shifted the concentration-response curve of isoprenaline to the right at both 10 and 100 µM concentration (Fig. 2) and Ec_{50} values were reduced significantly in both treatments (Table I).

TABLE I : Effect of trypan blue (TB) on the agonists (isoprenaline and dobutamine) induced positive inotropy on the isolated guinea-pig atrium. EC_{50} was determined as the concentration which produces half of maximum response in each condition. EC_{50} values are reported as mean ± SEM.

	EC_{50} [M]		
Agonist	*Control*	+10 µM TB	+100 µM TB
Isoprenaline	$1.1316(\pm 0.19)\times10^{-8}$	$2.4611(\pm0.60)\times10^{-8}$*	$5.3779(\pm1.24)\times10^{-8}$**
Dobutamine	$1.777(\pm0.59)\times10^{-6}$	$1.0375(\pm0.24)\times10^{-5}$*	$1.2053(\pm0.06)\times10^{-5}$**

*Significantly different from control ($P< 0.05$);
**Significantly different from control ($P< 0.01$)

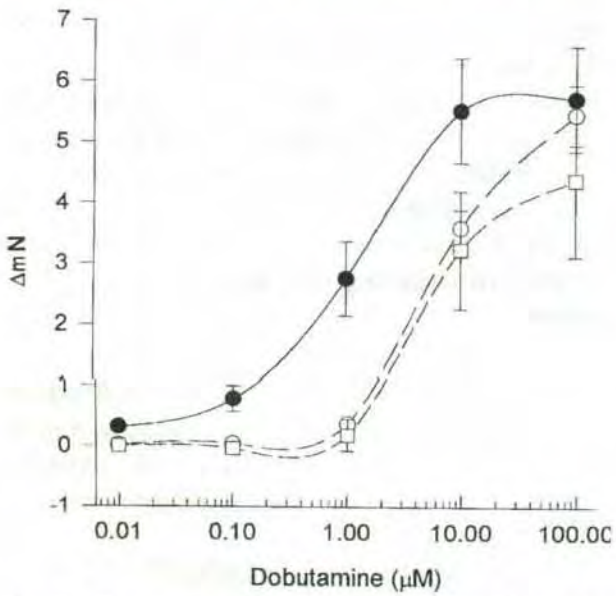

Fig. 1: Concentration-response curves for dobutamine in the absence or presence of trypan blue in electrically paced guinea-pig left atria. Vertical axis is the increase in the force of contraction (Δ mN).
(●)Dobutamine alone, (O) Dobutamine in presence of 10 µM trypan blue (□) Dobutamine in presence of 100 µM of trypan blue. Each point is the mean of at least six experiments and vertical bar indicates SEM.

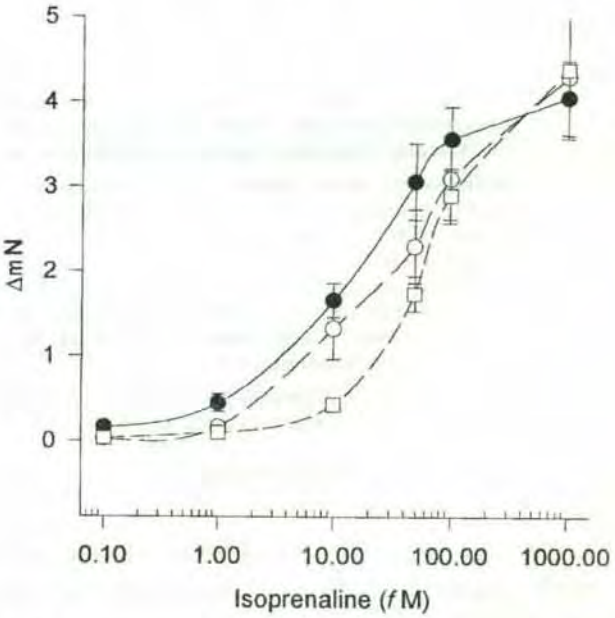

Fig. 2: Concentration-response curves for isoprenaline in the absence or presence of trypan blue in electrically paced guinea-pig left atria. Vertical axis is the increase in the force of contraction (Δ mN).
(●)Isoprenaline alone; (O) Isoprenaline in presence of 10 µM trypan blue; (□) Isoprenaline in presence of 100 µM of trypan blue. Each point is the mean of at least six experiments and vertical bar indicates SEM.

Effect of trypan blue on the action of salbutamol

Trypan blue, significantly diminished positive inotropic action of salbutamol in a concentration dependent manner (Fig. 3).

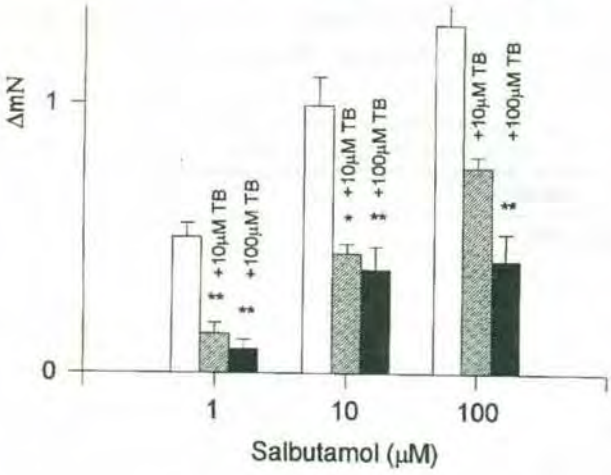

Fig. 3: Effect of trypan blue on the positive inotropic action of three different salbutamol concentration (1 μM, 10 μM and 100 μM), in the electrically stimulated guinea-pig left atrium.

Salbutamol alone (control, blank column), or in the presence of, 10 μM and 100 μM trypan blue (TB, hashed and black columns respectively). Each point is the mean of at least six experiments and vertical bar indicates SEM. The positive inotropic effects were compared in the presence and absence of trypan blue using paired Student's t-test.

*Significantly different from control ($P < 0.05$)
**Significantly different from control ($P < 0.01$)

DISCUSSION

It has been reported that compounds with spaced anionic moieties on an amphipathic structure could act directly at the site of receptor-G protein coupling to prevent the interaction between these two proteins and reduce receptor affinity for agonist (3, 11). Biochemical studies have shown that trypan blue, a member of this group has the ability to uncouple muscarinic, as well as α_2–, α_1–, and β_2–adrenoceptors from G–proteins (3, 11, 21, 22). In whole tissue preparation it is shown that trypan blue can block p_{2x}–purinoceptors in rat vas deferens (18), and inhibit the action of TSH on the thyroid follicular cells (23). It is reported that action of trypan blue on guinea-pig ileal muscle is rather specific (19). While, it has no effect on the response to acetylcholine, it prevents the relaxation effect of salbutamol, a β_2 agonist (19). Our results also showed that trypan blue can interfere with the positive inotropic action of β_2– and β_1–adrenoceptor agonists (isoprenaline, dobutamine and salbutamol) in isolated guinea-pig atria. It significantly increase EC_{50} values of isoprenaline and dobutamine (Fig. 1 and Table I). Agonist binding to the β–adrenoceptors stimulate adenylyl cyclase by activation of Gs protein and, cause a positive inotropic effect (24). Thus in accordance to biochemical studies (3, 11) trypan blue may act on the receptor G-protein coupling site and inhibit ligands' effects.

These results in accordance with others (17–19, 23) show, that trypan blue is a good tool for studying receptor-G protein interaction in whole tissue preparations.

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