## Communications to the Editor

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# THE EFFECTS OF TRAXANOX SODIUM ON CYCLIC AMP PHOSPHODIESTERASE AND THE CONCENTRATION OF CYCLIC AMP IN THE PLASMA OF RATS

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Traxanox sodium salt pentahydrate (9-chloro-5-oxo-7-(1H-tetrazol-5-yl)-5H-[1]benzopyrano[2,3-b]pyridine sodium salt pentahydrate, Code No. Y-12,141) (traxanox sodium) inhibited cyclic AMP phosphodiesterase in the lungs and hearts of rats. The inhibitory effect of traxanox sodium was more potent than that of both theophylline and sodium cromoglycate, but was less potent than that of papaverine. In the low Km (2 μM) enzyme of lungs the inhibition by traxanox sodium was competitive and its inhibition constant (Ki) was 66.7 μM. Intraperitoneal injection of traxanox sodium (100 mg/kg) synergistically enhanced the isoproterenol-induced increase of cyclic AMP in the plasma of rats.

KEYWORDS——traxanox sodium; antiallergic agent; cyclic AMP phosphodiesterase; lung; heart; cyclic AMP in plasma; rat; cyclic AMP phosphodiesterase inhibitor

Antigen-IgE-antibody interaction causes a release of chemical mediators such as histamine from human lungs,[1] human leucocytes,[2] and rat mast cells.[3] This release is inhibited by cyclic AMP analogues and beta-adrenergic agonists or prostaglandins, both of which activate adenylate cyclase, and also by cyclic AMP phosphodiesterase inhibitors.[4~7] Previous reports from our laboratories described traxanox sodium salt pentahydrate (Code No. Y-12,141) (traxanox sodium) as a potent antiallergic agent which markedly inhibits both IgE-mediated anaphylaxis and IgE-mediated histamine release from rat mast cells.[3,8,9] The present study was carried out to evaluate the inhibitory effects of traxanox sodium on cyclic AMP phosphodiesterase activities in vitro and in vivo in comparison with papaverine, disodium cromoglycate and theophylline, which are known to be cyclic AMP phosphodiesterase inhibitors.[7]

Reagents used were as follows; [8-$^3$H] cyclic AMP (30 Ci/m mol) and cyclic AMP assay kit (TRK-432) (from Amersham), cyclic AMP, snake venom (ophigus hannah), theophylline and papaverine (from Sigma Co.), AG 1x2 (200-400 mesh) (from Bio-Rad). Traxanox sodium salt pentahydrate (traxanox sodium) and disodium cromoglycate (DSCG) were synthesized in our laboratories. The other reagents were of guaranteed grade. Male donryu rats (140-160 g) were purchased from Nippon Rat KK.

Cyclic AMP phosphodiesterase activities were determined as described elsewhere.[10,11]

The ventricles and lungs of rats were homogenized in a Potter-Elvehjem type

homogenizer with 9 volumes of 0.04 M Tris buffer (containing 0.25 M sucrose, 5 mM 2-mercaptoethanol and 5 mM $MgCl_2$, pH 7.5). The homogenates were centrifuged at 105,000 g for 1 h at 4°C. The supernatant was used for the assay of cyclic AMP phosphodiesterase activities. A mixture consisting of 0.05 ml of this enzyme solution, 0.25 ml of 0.06 M Tris buffer (containing 10 mM $MgCl_2$, 2 mM 2-mercaptoethanol and 200 mM NaCl, pH 7.5), 0.05 ml of 0.1% bovine serum alubmin, 0.05 ml of test solution and 0.1 ml of [8-$^3$H] cyclic AMP was incubated for 10 min at 37°C. The reaction was stopped by immersion in boiling water for 2 min. After chilling with tap water, the mixture was incubated with 0.05 ml of snake venom (50-100 μg) for 15 min at 30°C. One ml of resin (3 volumes of $H_2O$; 1 volume of resin) and 0.05 ml of 1 mM adenosine were added, followed by incubation for 10 min at 30°C, and centrifugation at 1,200 g for 10 min. The concentration of radioactivity in the supernatant was determined with a liquid scintillation counter. The results are shown as % inhibition.

The concentration of cyclic AMP in plasma was assayed as described previously.[12] Compounds tested were intraperitoneally (i.p.) or intravenously (i.v.) injected into rats anesthetized with pentobarbital (80 mg/kg, i.p.). After 45 min, the blood obtained from the inferior vena cava of the rats was mixed with 1/100 volume of 0.5 M EDTA and centrifuged at 1,200 g for 15 min. The concentration of cyclic AMP in the plasma obtained by centrifugation was determined with the assay kit. Isoproterenol

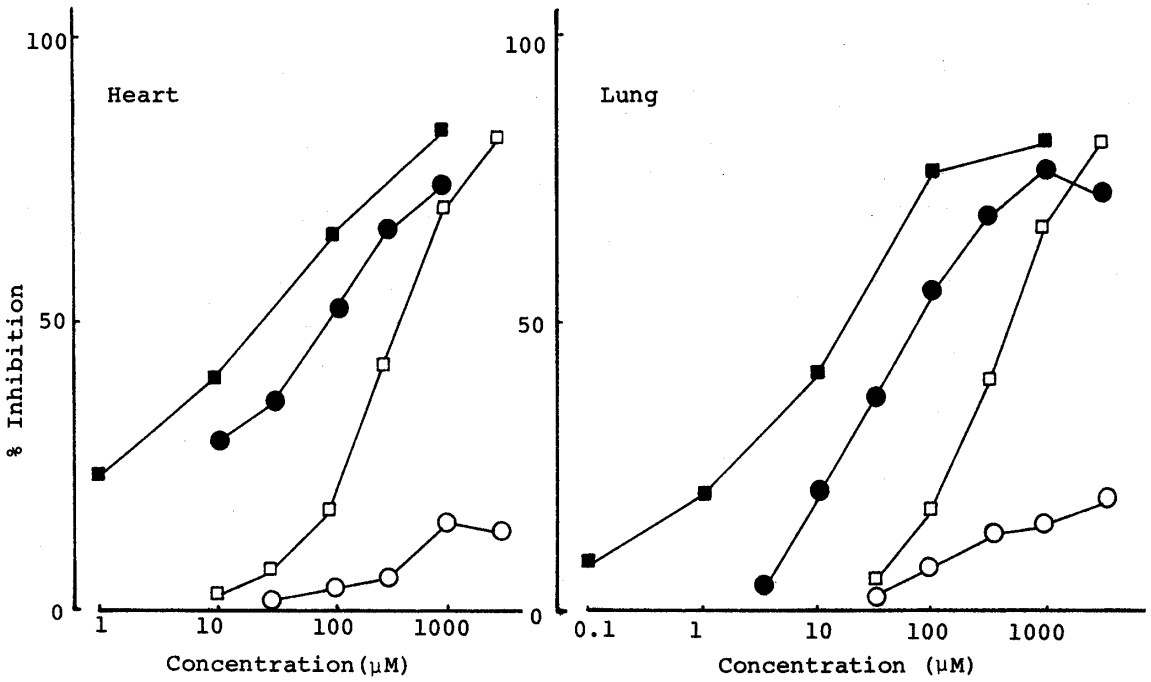


Fig.1. Inhibitory Effect of Traxanox Sodium on Cyclic AMP Phosphodiesterase Activity in Lungs and Hearts of Rats

Enzyme activity was determined at a concentration of 2 μM cyclic AMP in the lung and 1 μM cyclic AMP in the heart. The data shown are the means of triplicate assays.

●; traxanox sodium, ○; disodium Cromoglycate, □; theophylline, ■; papaverine.

was injected i.p. into the rats 15 min before collecting the blood.

Traxanox sodium inhibited the cyclic AMP phosphodiesterase activities of the hearts and lungs of the rats in vitro as shown in Fig.1. At 50% inhibition in both tissues the order of inhibitory potency was as follows: papaverine>traxanox sodium>theophylline>DSCG. Kinetics studies of cyclic AMP phosphodiesterase from the lungs of rats showed two apparent Km values, a low (2 μM) and a high (200 μM) (data not shown). Traxanox sodium competitively inhibited the low Km enzyme with an inhibition constant, Ki, 66.7 μM (data not shown).

Table I. Effect of Traxanox Sodium on the Concentration of Cyclic AMP in Rat Plasma

| Compounds | cyclic AMP pmol/ml of plasma |
|---|---|
| Untreated control | 14.0±0.5[1)] |
| Traxanox sodium (3 mg/g, i.p.) | 17.5±1.5 |
| Isoproterenol (0.1 mg/kg, i.p.) | 228.0±11.7[a)] |
| Traxanox sodium (3 mg/kg, i.p.) + Isoproterenol (0.1 mg/kg, i.p.) | 217.4±18.9 |
| Untreated control | 31.0±5.4 |
| Traxanox sodium (100 mg/kg, i.p.) | 104.4±4.1[a)] |
| Isoproterenol (0.1 mg/kg, i.p.) | 190.1±22.2[a)] |
| Traxanox sodium (100 mg/kg, i.p.) + Isoproterenol (0.1 mg/kg, i.p.) | 416.2±17.9[b)] |
| Untreated control | 18.3±1.3 |
| Theophylline (100 mg/kg, i.p.) | 154.4±14.3[a)] |
| Isoproterenol (0.1 mg/kg, i.p.) | 235.5±28.4[a)] |
| Theophylline (100 mg/kg, i.p.) + Isoproterenol (0.1 mg/kg, i.p.) | 957.4±68.2[b)] |
| Untreated control | 24.6±2.1 |
| DSCG ( 3 mg/kg, i.v.) | 23.3±0.6 |
| Isoproterenol (0.1 mg/kg, i.p.) | 241.6±15.1[a)] |
| DSCG (3 mg/kg, i.v.) + Isoproterenol (0.1 mg/kg, i.p.) | 244.2±22.5 |

1) Mean±S.E. (n=4∿5).
a) Statistical significance from control ($P<0.05$).
b) Statistical significance from isoproterenol alone ($P<0.05$).

It has been reported that the concentration of cyclic AMP in plasma is increased by beta-adrenergic agonists and this increase is synergistically enhanced by theophylline, a cyclic AMP phosphodiesterase inhibitor.[13] As shown in Table I, i.p. injection of traxanox sodium (100 mg/kg) increased the concentration of cyclic AMP in plasma. The isoproterenol-induced increase of cyclic AMP in plasma was synergistically activated by pretreatment with traxanox sodium (100 mg/kg, i.p.). This shows that traxanox sodium has an inhibitory effect on cyclic AMP phosphodiesterase in vivo.

Traxanox sodium has a greater inhibitory effect on cyclic AMP phosphodiesterase in vitro than theophylline, but the former is less effective than the latter in enhancing the isoproterenol-induced increase of cyclic AMP in plasma. The reason for this discrepancy is not clear. Saitoh et al.[13] have suggested that a large portion of the increase of cyclic AMP in plasma induced with beta-adrenergic agonists may come from skeletal muscles. Although traxanox sodium is rapidly absorbed after i.p. injection into rats and is resistant to metabolic degradation, its concentration in muscular tissue was small.[14] This low concentration in muscular tissue may be related to its weak effect in vivo compared with theophylline.

Oral doses of traxanox sodium producing antiallergic effects in rats ranging from 2.5 to 250 mg/kg, depending on experimental models.[15]

In summary, the present experiments indicate that the inhibitory activities of traxanox sodium on cyclic AMP phosphodiestrase may partially contribute to its inhibition of the release of allergic mediators.

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