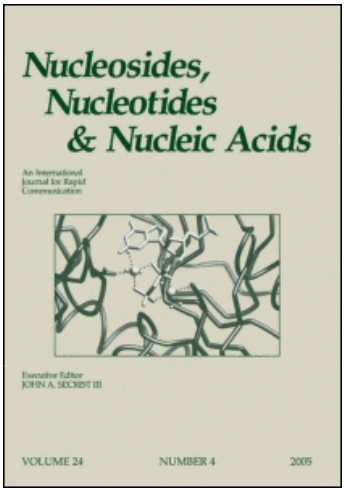


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# Autoradiographical Studies of in Vitro and Chronic in Vivo Effects of Propentofylline on Adenosine $A_1$ and $A_2$ Receptors and NBMPR-Sensitive Nucleoside Transporters

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# AUTORADIOGRAPHICAL STUDIES OF IN VITRO AND CHRONIC IN VIVO EFFECTS OF PROPENTOFYLLINE ON ADENOSINE $A_1$ AND $A_2$ RECEPTORS AND NBMPR-SENSITIVE NUCLEOSIDE TRANSPORTERS.

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Propentofylline is a novel xanthine that has been shown to limit the extent of neuronal damage induced by cerebral ischemia in gerbils (DeLeo et al., 1987). This is in contrast to other xanthines, including caffeine and theophylline, that increase the extent of damage (Rudolphi et al., 1987; Dux et al., 1989). Propentofylline has been demonstrated to decrease adenosine uptake into human erythrocytes (Fredholm and Lindström, 1986), and to increase extracellular concentrations of adenosine in ischemic brain (Andine et al., 1990). Therefore, it was proposed that this compound provides protection in cerebral ischemia, in part, by adenosine receptor stimulation due to increased endogenous adenosine levels.

One purpose of this study was to determine the potencies of propentofylline, and its major metabolite, A72 0287, for adenosine $A_1$ and $A_2$ receptors and NBMPR-sensitive nucleoside transport sites. We also investigated whether chronic propentofylline treatment altered the density or GTP-sensitivity of adenosine receptor agonist binding sites, or the density of NBMPR-sensitive nucleoside transport sites.

## METHODS

A. Acute treatment with propentofylline

$A_1$ Receptors: Coronal sections (10 $\mu$m) from rat brain were preincubated with adenosine deaminase (2 I.U./ml) for 30 min at 37 °C, incubated for 2 hours with [$^3$H]CHA (2.3 nM) and graded concentrations of propentofylline or A72 0287, washed twice with ice-cold with buffer (TRIS-HCl, 170 mM, pH 7.4), rinsed with ice-cold distilled water, dried overnight and apposed to [$^3$H] sensitive film for 3 weeks. Autoradiograms were analyzed using a computer image analysis system.

$A_2$ Receptors: Sections were processed as above, except the sections were washed with buffer containing 10 mM $Mg^{++}$ prior to incubation with [$^3$H]CGS. The incubation medium contained 2.4 nM [$^3$H]CGS, 10 mM $Mg^{++}$, and graded concentrations of propentofylline or A72 0287.

Nucleoside Transporters: Sections were incubated for 60 min with 1 nM [$^3$H]NBMPR and graded concentrations of propentofylline or A72 0287 and then processed as above.

B. Chronic treatment with propentofylline.

Propentofylline, 40 mg/kg/day, was administered to gerbils for four weeks, then withdrawn one day before sacrifice. Sagittal sections (10 $\mu$m) from 5 control and 5 treated animals were used for receptor autoradiography studies as described

| TABLE 1. | $A_1$ ($\mu$M) | $A_2$ ($\mu$M) | Transpoter ($\mu$M) |
|---|---|---|---|
| propentofylline | 97 (56-167) | 147 (107-403) | 223 (132-376) |
| A72 0287 | 92 (18-159) | 56 (5-125) | 61 (36-103) |

above, except without propentofylline or A72 0287. GTP, in concentrations of 0, 0.5, 5 or 100 $\mu$M, was added to receptor agonist incubation media.

## RESULTS

Propentofylline and A72 0287 completely inhibited binding of all three radioligands. Inhibition constants ($k_i$ values and 95% confidence intervals) for inhibition of [$^3$H]CHA binding in the stratum radiatum of the CA1 field of the hippocampus, and for [$^3$H]CGS and [$^3$H]NBMPR binding in the caudate putamen are listed in Table 1.

Binding site distribution and GTP-sensitivity of adenosine receptor agonists was determined by quantitative autoradiography of sagittal sections from control and chronic propentofylline treated gerbils. Twenty regions were studied for differences in [$^3$H]CHA binding. No differences in binding distribution in the absence or in the two higher concentrations of GTP were detected. In the presence of 0.5 $\mu$M GTP, three layers in the CA1 field of the hippocampus had greater [$^3$H]CHA binding in the propentofylline treated animals. [$^3$H]CGS binding in the caudate putamen was analyzed and found to be similar in the two groups of animals in the absence or in the presence of GTP (0.5, 5, or 100 $\mu$M). The distribution of specific [$^3$H]NBMPR binding sites was analyzed in 11 regions in the two groups of animals and no differences between the groups were apparent.

## DISCUSSION

Propentofylline and A72 0287 were able to inhibit completely binding of [$^3$H]CGS and [$^3$H]CHA, adenosine $A_2$ and $A_1$ receptor agonists, respectively, and of [$^3$H]NBMPR, a nucleoside transport inhibitor. No marked selectivity for any of the systems studied was apparent. While xanthines are well-known competitive antagonists at adenosine receptors, direct interactions with nucleoside transport sites are generally not observed and may explain the particular profile of action of propentofylline as previously suggested (Fredholm and Lindström, 1986).

Propentofylline, 10 mg/kg i.p., has previously been shown to reduce cell damage associated with cerebral ischemia (DeLeo et al., 1987; Dux et al., 1990). However, in this study, chronic oral administration of propentofylline, at the dose of 40 mg/kg/day for 4 weeks, did not alter binding distribution of any of the radioligands used. GTP-sensitivity of agonist binding to adenosine $A_1$ or $A_2$ receptors was also unaffected; thus this treatment apparently did not alter G-protein coupling of the receptors.

In conclusion, it appears that propentofylline and its metabolite A72 0287 have affinity for adenosine $A_1$ and $A_2$ receptors and for NBMPR-sensitive nucleoside transporters. However, chronic administration of propentofylline (40 mg/kg) did not lead to adaptive changes in distribution or GTP-sensitivity of these systems.

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