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THEOPHYLLINE INHIBITION OF RENAL AND CEREBRAL NUCLEOSIDE FORMATION

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1 Theophylline inhibits the enzymatic formation of purine nucleosides, among these adenosine (dephosphorylated adenosine 5′-monophosphate), in kidney and brain of the rat.

2 Some pharmacological effects of theophylline on regional blood flow and electrophysiological activity of the nervous system may be caused by inhibition of the endogenous formation of adenosine.

Introduction

Theophylline is a general stimulant of the central nervous system and intoxication results in centrally triggered convulsions (Ritchie, 1970; Warszawski, Gorodischer & Kaplanski, 1978; Helliwell & Berry, 1979; Gal, Roop, Robinson & Erkan, 1980). Furthermore, theophylline reduces the cerebral blood flow (Gottstein & Paulson, 1972) but increases the renal blood flow (Osswald, Schmitz & Kemper, 1977). Theophylline decreases the survival of mice exposed to hypoxia and increases the cerebral concentration of adenosine 5′-monophosphate (AMP) (Thurston, Hauhart & Dirgo, 1978).

Adenosine has a pronounced inhibitory effect on the activity of the nervous system, and theophylline is a potent antagonist of this effect of adenosine (Phillis & Edstrom, 1976; Hedquist, Fredholm & Ölundh, 1978; Hollins & Stone, 1980). Further, theophylline *per se* has the opposite effect to adenosine on the release of noradrenaline from electrically stimulated adrenergic nerves (Hedquist *et al.*, 1978). Adenosine is a potent vasodilator and increases the blood flow in the brain (Berne, Rubio & Curnish, 1974) but in the kidney adenosine is a vasoconstrictor and decreases blood flow (Osswald *et al.*, 1977).

Theophylline inhibits phosphodiesterase (EC 3.1.4.17) and consequently increases cyclic AMP (Butcher & Sutherland, 1962; Drummond & Yamamoto, 1971). Adenosine also increases cyclic AMP, e.g. in brain tissue (Sattin & Rall, 1970; Rall, 1972). The effects on the cyclic nucleotide system of theophylline and adenosine can hardly explain their antagonism.

Adenosine (dephosphorylated AMP) and other nucleosides are formed by the membrane enzymes 5′-nucleotidase (EC 3.1.3.5) and/or the 5′-nucleotidase activity of alkaline phosphatase (EC 3.1.3.1). The 5′-nucleotidase activity of some purified alkaline phosphatases is inhibited by theophylline (Jensen, 1979), and the different mononucleotides seem to be dephosphorylated by electrophoretically identical enzyme molecules (Jensen, Iversen & Hägerstrand, 1980).

In this paper the effect of theophylline on the enzymatic nucleoside formation in the kidney and the brain of the rat is described.

Methods

Kidneys and brains from 20 adult male Wistar rats, anaesthetized with ether, were used for the enzyme preparations. To avoid admixture of enzymes from the blood cells, the organs were thoroughly perfused *in situ* with physiological saline to which heparin had been added (1000 iu/l). This was carried out via infusion into the left ventricle of the heart with subsequent discharge of the perfusion fluid through the right atrium. After the perfusion, the organs were homogenized in 10 volumes of sucrose (0.25 M)-$MgCl_2$ (5 mM). Then the homogenate was centrifuged at 10000 *g* for 2 h, and the supernatant dialyzed for 18 h at 4°C against 10 litres of a solution of Tris buffer (5 mM, pH 7.4)-$MgCl_2$ (5 mM) with constant stirring. The 5′-nucleotidase activity in this dialysate was studied.

The substrates were adenosine, inosine and guanosine 5′-monophosphate (AMP, IMP and GMP). The preparation of the solutions of substrates and of theophylline was carried out as described previously, as was the incubation procedure (Jensen, 1979). All the incubations were carried out at 37°C at pH 7.4; 250 μl of the kidney or the brain dialysate were incubated in each experiment with a substrate solution with or without theophylline added. The reaction was stopped after the desired incubation time (30, 60, 90 or 120 min) by the addition of 250 μl of trichloroacetic acid, and the liberated phosphate determined colorimetrically. The amount per minute

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Table 1 The effect of theophylline on the dephosphorylation of purine mononucleotides by phosphomonoesterases from kidney and brain of the rat

	Substrate	*Control*	*Theophylline* 1.0 mM	5.0 mM	10.0 mM
Kidney	AMP	10.8 ± 0.3	9.1 ± 0.3 = 16%***	8.1 ± 0.2 = 25%***	6.3 ± 0.4 = 42%***
	IMP	5.7 ± 0.2	5.2 ± 0.0 = 9%***	4.9 ± 0.1 = 14%***	4.4 ± 0.1 = 23%***
	GMP	6.1 ± 0.4	5.6 ± 0.1 = 8%*	5.4 ± 0.1 = 11%**	5.0 ± 0.2 = 18%***
Brain	AMP	4.4 ± 0.2	3.9 ± 0.2 = 11%**	3.5 ± 0.2 = 21%***	2.6 ± 0.3 = 40%***
	IMP	2.4 ± 0.1	2.1 ± 0.2 = 13%*	1.8 ± 0.2 = 24%***	1.4 ± 0.1 = 42%***
	GMP	2.7 ± 0.1	2.3 ± 0.3 = 14%*	2.1 ± 0.0 = 24%***	1.8 ± 0.1 = 34%***

The phosphomonoesterase activity was determined as the amount of phosphate liberated following the incubation of an enzyme preparation (250 µl) with a purine mononucleotide at 37°C at pH 7.4. Each value (µM phosphate liberated per min) is the mean ± 2 s.e.mean of 16 determinations = the coefficient of slope for the line describing the relationship between incubation time and amount of liberated phosphate. This relationship was in all cases linear ($r = 0.97-1.00$). The inhibition in per cent and the level of significance are shown also: $*P<0.05$; $**P<0.01$; $***P<0.001$. The comparisons were made by Student's *t* test for paired data.

was determined as the coefficient of slope for the line describing the relationship between the duration of incubation and the amount of liberated phosphate (Jensen, 1979). Sixteen experiments were performed for each substrate at each concentration of theophylline.

Results

As shown in Table 1, theophylline inhibited the dephosphorylation of all the purine mononucleotides studied.

Discussion

The hypothesis has been put forward, that the electrophysiological activity of the nervous system is accompanied by an endogenous liberation of adenosine from the nerve cells, which subsequently reduces the neurone activity (Phillis & Edstrom, 1976). In agreement with this, electric pulses and various depolarizing stimuli liberate adenosine and other dephosphorylated purines from rat hypothalamic synaptosomes (Fredholm & Vernet, 1979). Another hypothesis suggests that endogenous adenosine plays an important role in the regulation of regional blood flow (Haddy, 1977), e.g. in the heart (Berne, 1963), in the kidney (Osswald, *et. al.*, 1977) and in the brain (Berne *et al.*, 1974).

The pharmacological effects of theophylline are opposite to the effects of adenosine and the inhibition of the enzymatic formation of adenosine by theophylline seems to be compatible with the adenosine hypotheses. Further, theophylline increases cerebral AMP and ADP concentrations without changing the ATP concentration (Thurston *et al.*, 1978). The increased AMP concentration may be caused by a decreased dephosphorylation of AMP. Unfortunately the adenosine level was not measured.

In certain neurones an adenosine-like effect of morphine has recently been demonstrated, and aminophylline antagonized this effect (Perkins & Stone, 1980). It is not yet known, however, if morphine increases the enzymatic formation of adenosine. Few studies of the possible effects of nucleosides other than adenosine have been made. However, inosine (dephosphorylated IMP) inhibits the electrophysiological activity of cultured mouse spinal neurones and interacts with benzodiazepine (Mac Donald, Barker, Paul, Marangos & Skolnick, 1979).

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