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ANTIPORPHYRIC ACTIVITY OF THEOPHYLLINE IN NORMAL AND ALLYLISOPROPYLACETAMIDE-TREATED RATS

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Abstract—Theophylline decreases the levels of 5-aminolevulinic (ALA) synthetase in allylisopropylacetamide(AIA)-treated rats. Since theophylline increases cell cAMP levels this result suggests cAMP involvement in ALA synthetase repression. Theophylline in AIA-treated rats also protects the levels of cytochrome P-450 which reflect the values of total heme, the natural repressor of ALA synthetase activity.

Allylisopropylacetamide (AIA) administration has been reported to depress NADH oxidase activity [1], to increase the NADH/NAD^+ ratio [2] and to impair the terminal oxidation [3]. Inhibition of NADH oxidation can result in decreased cellular ATP [3]. A fall in ATP levels is associated with an increase of NADH values in rats treated with AIA [4, 5]. Since cyclic adenosine monophosphate (cAMP) synthesis from ATP is catalyzed by adenyl cyclase [6], a lowering of ATP levels, caused by AIA injection may lead to a secondary fall of cAMP. Recently we reported that AIA administration to rats caused a marked decrease of liver cyclic AMP levels, associated with an increase of 5-aminolevulinic acid (ALA) synthetase activity [7]. Furthermore, dibutyryl cyclic AMP (DBcAMP), a drug mimicking cAMP [8] lowered ALA synthetase activity in rats even if treated with AIA [7].

These observations suggest a role of cAMP levels in the repression of ALA synthetase activity. Therefore drugs capable of increasing endogenous cAMP could display antiporphyric activity. If so, it seemed of interest to test this hypothesis in experimentally induced porphyria.

Theophylline, a drug capable of increasing tissue cAMP levels [9] was administered to AIA-treated rats and its effects on ALA synthetase activity, the rate limiting enzyme of porphyrin biosynthesis [10] and on cytochrome P-450 levels which reflect the values of total heme [5], the natural repressor of ALA synthetase activity [12] were evaluated.

MATERIALS AND METHODS

AIA was supplied by Hoffman-LaRoche (Milano). Materials and reagents used in these experiments were obtained from BDH (Milano).

Female Wistar rats weighing 100 ± 10 g were fasted for 24 hr before injecting the porphyrogenic drug and kept without food for the duration of the experiments. Each experimental group contained 6 rats. One group of animals acted as controls, while another group was injected subcutaneously with AIA (400 mg/kg) on two consecutive days. At the concentration of 4%, AIA was soluble in a mixture of water, polyethylene glycol and ethanol (60:30:10, by vol.). Other groups of animals were injected intraperitoneally with theophylline (20 and 80 mg/kg) or were treated with AIA and theophylline.

The control and theophylline groups were treated with the vehicle used for dissolving AIA. The dose of theophylline was divided into two equal parts and administered immediately, and 3 hr after the second injection of AIA.

A group of rats was sacrificed 6 hr after the second AIA injection. The livers were immediately removed and a portion was kept at 0°C for the immediate determination of 5-aminolevulinic acid (ALA) synthetase activity [13].

Another group of rats was killed 5 hr after the second AIA injection. The total liver was immediately removed, weighed, kept at 0° and perfused with cold 1.5% KCl. The microsomal fraction was prepared [14] and the cytochrome P-450 [15], and protein concentrations were determined [16].

RESULTS AND DISCUSSION

AIA administration stimulated ALA synthetase activity and theophylline decreased the levels of this enzyme in AIA treated rats (Table 1). The fact that theophylline, which increases cell cAMP levels [9],

Table 1. Effects of AIA, theophylline (20 mg/kg or 80 mg/kg) and combined treatment on liver ALA synthetase activity

Treatment	ALA synthetase activity	
	Expt. I	Expt. II
Controls	9.39 ± 2.27	13.32 ± 1.92
Theophylline (20 mg/kg)	7.61 ± 1.74	—
Theophylline (80 mg/kg)	—	7.27 ± 1.10
AIA	207.30 ± 8.64	271.81 ± 37.62
AIA + theophylline (20 mg/kg)	135.97 ± 10.32*	—
AIA + theophylline (80 mg/kg)	—	100.53 ± 10.15*

The results are expressed in nmole/hr/g wet liver and each value is the mean ± S.E. of six determinations.

* Significantly different from the animals treated with AIA alone, $P < 0.01$.

Table 2. Effects of AIA, theophylline (80 mg/kg) and combined treatment on liver wet weight, microsomal proteins and cytochrome P-450

Treatment	Number of animals	Liver wet wt (g/100 g body wt)	Microsomal proteins (mg/total liver per 100 g of body wt)	Microsomal cytochrome P-450 (nmoles/mg microsomal protein)	Microsomal cytochrome P-450 (nmoles/g of wet liver per 100 g body wt)
1 Controls	6	3.408 ± 0.092	55.6 ± 2.2	0.811 ± 0.026	11.13 ± 0.89
2 Theophylline	6	3.402 ± 0.017	52.9 ± 3.3	0.674 ± 0.038	10.49 ± 0.79
3 AIA	6	4.484 ± 0.124	79.7 ± 5.1	0.418 ± 0.033	7.78 ± 0.51
4 AIA + theophylline	6	4.470 ± 0.193	81.8 ± 3.7	0.566 ± 0.030	9.98 ± 0.46
Significance levels	1–2	N.S.	N.S.	$P < 0.02$	N.S.
	1–3	$P < 0.01$	$P < 0.01$	$P < 0.01$	$P < 0.01$
	2–4	$P < 0.01$	$P < 0.01$	$P < 0.05$	N.S.
	3–4	N.S.	N.S.	$P < 0.01$	$P < 0.01$

caused an inhibition of ALA synthetase activity supports the hypothesis of cAMP involvement in ALA synthetase repression [7, 17].

AIA also caused a loss of cytochrome P-450 and theophylline administration protected cytochrome P-450 from being destroyed in animals receiving AIA (Table 2).

Since cytochrome P-450 is a heme-bound protein and reflects the total heme levels [11] and since heme is the natural repressor of ALA synthetase activity [12], theophylline, by protecting heme from being destroyed, exerts a suppressive effect on ALA synthetase. It is not clear if the protective effect on heme, exerted by theophylline in AIA injected rats, is mediated through a decreased activity or rapid removal of the porphyrogenic drug, caused by cAMP, which is known to affect drug metabolism [18].

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