available in the literature. However, cyclic AMP at concentrations from 10^{-9} M to 10^{-3} M did not affect lung cyclase activity ($\leq 7\%$ change), as might have been expected if kinase activity regulated cyclase activity in these fractions (see also reference[4]). The results of this experiment suggest that cyclic nucleotides do not inhibit lung cyclase activity via effects on an associated protein kinase, and that adenosine analogs and cyclic nucleotides may inhibit cyclase activity by distinct interactions with the cyclase moiety.

Zusammenfassung. Substitution einer N^6-Aminogruppe eines zyklischen Nukleotids führt zu einer erhöhten Hemmwirkung des Nukleotids gegenüber Adenyl-Cyclase von Meerschweinchenlungen, während die N^6-Amino-Substition von Adenosin-Analogen eine herabgesetzte Inhibitionswirksamkeit gegenüber demselben Enzym zur Folge hat. Die experimentellen Daten führen zu dem Schluss, dass der Inhibitionsmechanismus gegenüber Cyclase für beide Verbindungstypen verschieden ist.

I. WEINRYB and I. M. MICHEL

Department of Biochemical Pharmacology, Squibb Institute for Medical Research, P.O. Box 4000, Princeton (New Jersey 08540, USA), 18 March 1975.

Influence of Methylxanthines on Aniline Disappearance and Metabolism in Rats

A change in microsomal enzyme activity may result in intoxication[1,2] or partial inactivation of drug action[3] and hence be hazardous to animals or man. Therefore, this study was undertaken to compare the in vivo and in vitro drug metabolism of methylxanthines which have been found to stimulate[4,5] or inhibit[6] microsomal enzyme activity when measured in vitro.

Materials and methods. For both assays, in vitro and in vivo measurement of microsomal enzyme activity, male rats weighing 315 $\pm$ 10 g were divided between 1 control and 8 test groups. Animals were pretreated with caffeine 150 mg/kg or 37.5 mg/kg, or theobromine 150 mg/kg or 37.5 mg/kg; instant tea, or instant coffee containing 75 mg/kg of caffeine. All these substances were diluted in water and administered per os daily for 3 days. 2 additional groups were injected i.p. either with 75 mg/kg of phenobarbital, a classic inducer[7] or 100 mg/kg of SKF 525-A a known inhibitor[8]. An in vivo and in vitro assay was also carried out using male rats (320 $\pm$ 20 g) which were pretreated with 75, 46 or 27 mg/kg of phenobarbital or 100, 56 or 32 mg/kg of SKF 525-A together with a control group.

The in vivo and in vitro measurements were made 24 h after the last administration of all test substances, except in the case of SKF 525-A measured after 1 h. To determine the microsomal metabolism in vivo, rats were injected i.p. with 50 mg/kg of aniline and after approxi-

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Table I. In vitro measurement of microsomal enzyme activity

	Aniline hydroxylase (pmoles p-amino phenol/mg protein/min incubation)	Cytochrome P-450 (nmoles/mg protein)
Control (water)	39.8 $\pm$ 4.0	0.831 $\pm$ 0.126
Theobromine (150 mg/kg)	67.1 $\pm$ 7.4[c]	0.783 $\pm$ 0.097
Theobromine (37.5 mg/kg)	38.8 $\pm$ 4.2	0.814 $\pm$ 0.051
Caffeine (150 mg/kg)	45.6 $\pm$ 3.9[a]	0.787 $\pm$ 0.163
Caffeine (37.5 mg/kg)	36.7 $\pm$ 5.6	0.833 $\pm$ 0.127
Instant coffee (containing 75 mg of caffeine/kg)	44.7 $\pm$ 4.6	0.824 $\pm$ 0.093
Instant tea (containing 75 mg of caffeine/kg)	51.7 $\pm$ 5.4[c]	0.830 $\pm$ 0.152
SKF 525-A (100 mg/kg once)	28.5 $\pm$ 5.7[b]	0.785 $\pm$ 0.095
Phenobarbital (75 mg/kg)	127.3 $\pm$ 15.9[c]	1.080 $\pm$ 0.106[b]

Theobromine, caffeine, instant coffee, instant tea and water for controls were administered per os daily for a 3-day pretreatment. Phenobarbital was injected i.p. daily for 3 days and SKF 525-A once only. Each group contained 8 male rats and mean values with confidence limits at 95% were given. Significant difference (t-test) between control and treated groups is indicated as: [a] $p < 0.05$; [b] $p < 0.01$; [c] $p < 0.001$.

Table II. Induction or inhibition of in vitro metabolism

	Aniline hydroxylase (pmoles *p*-amino phenol/mg protein/min incubation)	Cytochrome P-450 (nmoles/mg protein)
Control	39.3 ± 2.3	0.750 ± 0.046
Phenobarbital (27 mg/kg)	50.0 ± 4.9	0.870 ± 0.040
Phenobarbital (48 mg/kg)	56.2 ± 3.0[b]	0.982 ± 0.060[a]
SKF 525-A (32 mg/kg)	44.5 ± 4.7	0.786 ± 0.098
SKF 525-A (56 mg/kg)	34.8 ± 5.2	0.761 ± 0.074

Phenobarbital was injected i.p. daily for 3 days and SKF 525-A once only. Each group contained 6 male rats and mean values with confidence limits at 95% were given. Significance is given as: [a] $p < 0.05$; [b] $p < 0.01$.

mate diffusion equilibrium was established, aniline disappearance from the blood was measured. Microsomal enzyme activity in vitro was measured by a method using aniline as substrate[9] and by determining the cytochrome P-450 level[10]. The LOWRY[11] method was used for protein determination.

Results. In vitro measurement. In rats pretreated with a high dose of methylxanthines (caffeine or theobromine at 150 mg/kg) or instant tea (1 g/kg containing about 75 mg/kg of caffeine) the aniline hydroxylation rate was significantly increased. However, instant coffee at a dose of 1.25 g/kg (containing 75 mg/kg caffeine) or methylxanthines at a lower dose (37.5 mg/kg) did not change microsomal enzyme activity. The microsomal enzyme activity was increased 3-fold when a dose of 75 mg phenobarbital/kg was given (Table I), at a dose of 48 mg/kg a smaller but still significant increase ($p < 0.01$) was also observed. Practically no change in enzyme activity was observed when phenobarbital was given at 27 mg/kg (significant increase only for $p < 0.10$). SKF 525-A, however, only inhibited enzyme activity significantly when given to animals at its highest concentration (100 mg/kg).

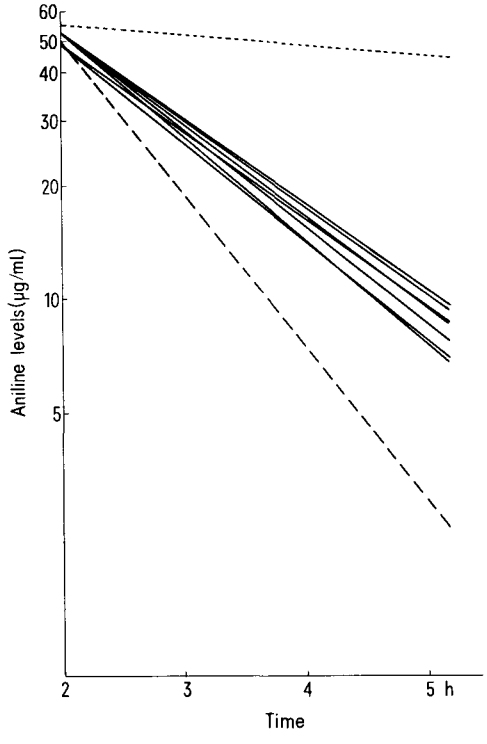

Fig. 1. In vivo measurement of microsomal enzyme activity. Theobromine, caffeine, instant coffee, instant tea and water for controls were administered per os daily over a 3-day pretreatment period. Phenobarbital was injected i.p. daily for 3 days and SKF 525-A once. Animals were then injected i.p. with 50 mg/kg of aniline. Aniline disappearance was measured using 6 male rats per group. Decline of aniline levels in serum over time was presented as regression lines on a semi-log scale. Significant difference between controls is indicated as $p < 0.01$[a] or NS if not significant. ————, Control, theobromine 150 or 37.5 mg/kg, caffeine 150 or 37.5, g/kg, instant tea or instant coffee containing 75 mg/kg of caffeine, all NS. - - - - - -, SKF 525-A 100 mg/kg[a]. – – – – – – –, Phenobarbital 75 mg/kg[a].

The cytochrome P-450 level remained unchanged for all the pretreatments except for phenobarbital, which caused a dose-dependent rise (Tables I and II). The significance between control and test groups were calculated using the *t*-test by plotting the treatment means with their 95% confidence limits on a line.

In vivo measurements. Since aniline disappearance was studied by measurements made at different times on the same animals, statistical multivariate analyses were applied. The 'Barlett' test showed that the 'within groups' dispersion matrixes were homogeneous. No significant differences in the rate of aniline disappearance in blood were detected, with respect to the control, and between groups pretreated with theobromine 150 or 37.5 mg/kg, caffeine 150 or 37.5 mg/kg, instant tea or instant coffee containing 75 mg/kg of caffeine. However, aniline disappearance was significantly faster or slower when rats were pretreated with a microsomal enzyme inducer (75 mg/kg or 46 mg/kg of phenobarbital) or an inhibitor (100 mg/kg or 56 mg/kg of SKF 525-A) respectively.

Discussion. Of the great number of publications investigating the influence of foreign compounds on microsomal enzyme metabolism measured in vitro, only a few were confirmed in vivo[1]. The in vivo metabolism represents a complex of interplays such as drug metabolism, degree of tissue binding, excretion etc.[12]. This

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explains why in the present study methylxanthines and instant tea stimulated microsomal enzyme activity measured in vitro, but since the level of aniline in the blood remained unchanged the in vivo metabolism was not altered. It was also shown that caffeine's shortening of sleeping time[13] was not due to the influence on drug metabolism but rather to an interaction at the brain level[14]. Furthermore, consumption of at least 6 cups of coffee or tea per day by humans did not induce liver microsomal enzyme activity[5].

Methylxanthines only caused an induction in vitro when given in concentrations of 75 mg/kg or higher, as confirmed by other workers[4,5]. In lower concentrations, methylxanthines did not change in vitro enzyme activity, however other authors[6] have claimed that caffeine given at 20 mg/kg inhibits microsomal enzyme activity. But their results were contradictory, since one of their substrates used indicated an inhibition, the other an induction. On the other hand, the cytochrome P-450 level was not changed by methylxanthines in the present study nor in the in vitro studies of the workers mentioned above[5,6].

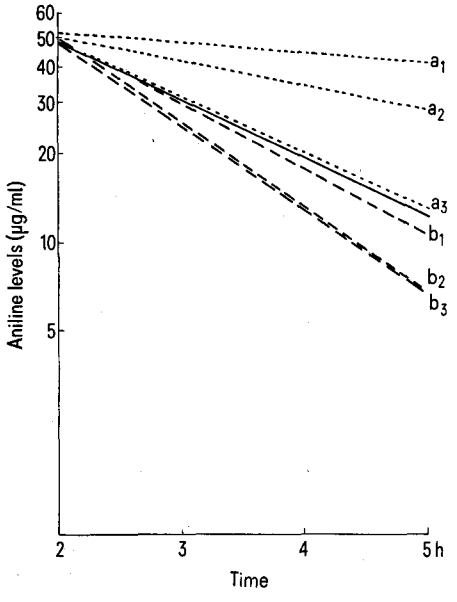

Fig. 2. Induction or inhibition of in vivo metabolism. Phenobarbital was injected i.p. daily for 3 days and SKF 525-A once. Animals were then injected i.p. with 50 mg/kg of aniline. Aniline disappearance from blood was measured using 6 male rats per group. Decline of aniline levels in serum was presented as regression lines on a semi-log scale. Significant difference between controls is indicated as $p < 0.01^a$ or NS if not significant. ————, Control group. - - - - - - -, a_1 SKF 525-A 100 mg/kg[a]; a_2 SKF 525-A 56 mg/kg[a]; a_3 SKF 525-A 32 mg/kg NS. – – – – – – –, b_1 Phenobarbital 27 mg/kg NS; b_2 Phenobarbital 48 mg/kg[a]; b_3 Phenobarbital 75 mg/kg[a].

Since cytochrome P-450 plays an important role in drug metabolism[15,16] and its level is normally increased by enzyme induction, it could be concluded that an induction of in vivo drug metabolism can only be expected if the cytochrome P-450 level is elevated. Especially since a dose-dependent induction of microsomal metabolism, caused by phenobarbital, showed a very good correlation between the P-450 level and the in vivo aniline metabolism. Whereas, in vitro aniline hydroxylation was always more pronounced than the in vivo metabolism.

However, no such correlation between in vivo and in vitro drug metabolism was observed when inhibition occurred, which suggests the involvment of a different mechanism. Aniline hydroxylation was only inhibited when SKF 525-A was administered to animals in a high concentration (100 mg/kg) which confirms other workers' findings[17]. But already at a lower concentration of SKF 525-A (46 mg/kg), in vivo aniline metabolism was inhibited which again agrees with other workers[18]. Provided that the microsomal enzyme system is relatively unspecific[19] it can be concluded that compounds, which induce in vitro drug metabolism might not always have an effect on microsomal metabolism when measured in vivo. Hence a normal coffee or tea consumption of 5 cups per day by a 70 kg man, resulting in an intake of about 7 mg/kg of caffeine and traces of theobromine, would not have harmful consequences through changes in microsomal enzyme activity.

Zusammenfassung. Eine Induktion der Mikrosomalenzyme der Leber, gemessen in vitro, wurde beobachtet, wenn hohe Dosen von Methylxanthinen an Ratten verabreicht wurde. Wenn den Versuchstieren gleich hohe Dosen von den obengenannten Substanzen verabreicht wurden, die Aktivität der arzneimittelabbauenden Enzyme jedoch in vivo gemessen wurde, so war kein Unterschied zur Kontrollgruppe festzustellen.

H. U. AESCHBACHER[20]

Nestlé Products Technical Assistance Company Limited, Biological Laboratories, CH-1350 Orbe (Switzerland), 21 January 1975.

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Characterization of Myxovirus Sialidase

In myxoviruses, the sialidase as well as haemagglutinin is localized on the outer-envelope of the virion particle. It has been reported that the antibody against virus sialidase was effective in preventing virus infection[1]. Recently the virus sialidase has been considered to play an important role in the process of virus multiplication[2,3]. The characterization of the sialidase will be useful for a better understanding of virus infection.

In the present communication, we compared the substrate specificity of the sialidase in several species of myxoviruses. Previously, we discovered an inhibitor against bacterial sialidase called siastatin[4]. The inhibitor