Monoamine oxidase inhibition by d-amphetamine in ganglia and nerve endings[1]

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Summary. The inhibition of monoamine oxidase (MAO) activity by d-amphetamine was measured in homogenates of cat superior cervical ganglion and nictitating membrane, using tyramine (TM) and noradrenaline (NA) as substrates. In both tissues, d-amphetamine was shown to be a competitive inhibitor of the oxidation of TM. The Ki for d-amphetamine, as a MAO inhibitor, was lower in the ganglia than in the peripheral nerve endings.

It has been shown that amphetamine is a competitive and reversible inhibitor of MAO in vitro and in vivo[2-5]. Recently, we demonstrated that the metabolism of ^{3}H-noradrenaline (^{3}H-NA) released by d-amphetamine from the nerve endings of the cat nictitating membrane differs widely from that of the ^{3}H-NA released from the cell bodies of the cat superior cervical ganglion[6]. To elucidate whether these dissimilarities are due to differences in MAO activity, we measured the potency of d-amphetamine as an inhibitor of TM and NA metabolism in both tissues.

Materials and methods. Cats of 2.0-4.0 kg b.wt and of either sex, were anesthetized with sodium pentobarbitone 35 mg/kg (i.p.). The tissues were excised and placed in a Petri dish with modified Krebs' solution previously bubbled with 95% O_2 and 5% CO_2. The composition of the Krebs' solution was as follows (millimolar concentration): NaCl, 118.0; KCl, 4.7; $CaCl_2$, 2.6; $MgCl_2$, 1.2; NaH_2PO_4, 1.0; $NaHCO_3$, 25.0; glucose, 11.1; ethylenediamine tetraacetic acid, 0.004 and ascorbic acid, 0.11.

The ganglia and the nictitating membrane were homogenized with an all-glass homogenizer in either 150 mM KCl or 150 mM sodium phosphate buffer (pH 7.3) respectively, when TM and NA were used as substrate. Determinations of enzyme activity were assayed in duplicate.

The method employed for the determination of MAO activity with TM as substrate, was carried out according to McCaman et al.[7]. The concentration of the substrate was 700 µM for ganglia and 200 µM for nictitating membrane. The assay was performed with 25 µl of the homogenate at 37 °C for 20 min.

MAO activity towards NA was assayed by the method described by Leitz and Stefano[8], and the concentration of the substrate was 50 µM. 25 µl of the homogenates were preincubated at 37 °C for 5 min, and then the assay mixture was incubated during 15 min.

d-Amphetamine sulphate was obtained from Smith Kline and French Laboratories. ^{3}H-Tyramine (sp. act. 10.78 Ci/mmole) and (−) ^{3}H-noradrenaline (sp. act. 5.71 Ci/mM) were obtained from New England Nuclear Corporation, Boston, USA. For the purification of (−) ^{3}H-NA, a solution (1 mCi/ml) of the amine was adjusted to pH 8.2 and poured over a short alumin column; the column was washed with 2 ml of sodium acetate and 2 ml of water. ^{3}H-NA was eluted with 3 ml of 0.2 N acetic acid. Protein concentrations were determined according to Lowry et al.[9]. Statistical calculations were performed according to conventional procedures[10].

Results. The apparent K_M for TM, determined from Lineweaver-Burk plots, was 210 µM in the nictitating membrane and 769 µM in the superior cervical ganglion. As can be seen in the figure, d-amphetamine inhibited competitively the oxidation of TM by MAO in both tissues.

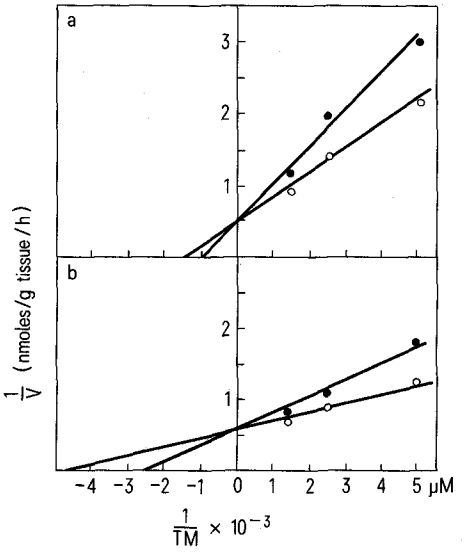
The Ki for d-amphetamine for inhibition of MAO in the superior cervical ganglion was almost 5 times lower than in the nictitating membrane. Consequently the IC_{50} for d-amphetamine to inhibit TM and NA deamination was also lower in ganglia than in nictitating membrane.

Discussion. Recently, we have shown that the potency of d-amphetamine for releasing ^{3}H-NA was higher in the nerve terminals than in the cell body. Moreover, the neurotransmitter released by d-amphetamine was metabolized to a greater extent in the cat superior cervical ganglion than in the nictitating membrane[8]. Differences between the 2 tissues in the potency of d-amphetamine to inhibit MAO could explain the faster metabolic degradation of the neurotransmitter released from the neuronal body. Contrary to what was expected, the present results showed that the Ki for d-amphetamine was almost 5 times lower in the superior cervical ganglion than in the nictitating membrane, indicating a greater potency of d-amphetamine as a MAO inhibitor of TM oxidation in the neuronal cell body than in the nerve terminals. Since the Ki of a competitive

Ki and IC_{50} for the inhibition of MAO by d-amphetamine

Tissue	Ki (µM)	IC_{50} ^{3}H-TM (µM)	^{3}H-NA (µM)
Superior cervical ganglion	19±3 (5)	37.2±6.0 (5)	3.4±0.6 (5)
Nictitating membrane	106±19 (5)*	206.2±38.1 (5)	15.1±2.2 (5)

Superior cervical ganglion and nictitating membrane were assayed individually. IC_{50}-values were determined graphically by probit transformation. Ki-values were obtained by dividing IC_{50} by $(1+S/K_M)$. ^{3}H-TM, ^{3}H-tyramine; ^{3}H-NA, ^{3}H-noradrenaline. Values are mean±SEM, in parentheses number of experiments. *$p<0.005$.



Lineweaver-Burk plots for cat superior cervical ganglion MAO (a) and for nictitating membrane MAO (b) with tyramine as substrate. -○- control; -●- in the presence of 10^{-4} M d-amphetamine. Each point represents the mean value obtained from duplicate determinations.

inhibitor is independent of the substrate[11,12], a similar difference should be mantained with NA.
In a previous paper, we reported that the 2 forms of MAO, type A and type B coexist in the cat superior cervical ganglion and nictitating membrane and that clorgyline has a greater potency for inhibiting type A in the nerve terminals than in the cell body[13]. Since TM is a substrate not allowing separate assay of the activity of both types of MAO, we also used NA. Under these conditions, d-amphetamine also showed a greater potency for inhibiting deamination in the superior cervical ganglion.
Although the present results do not explain the differences in the metabolism of the transmitter released by d-amphetamine from the superior cervical ganglion and from the nictitating membrane, they point to further differences between the MAO of the cell body and the nerve endings.

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Effects of polycyclic hydrocarbons on the induction of chromosomal aberrations in absence of an exogenous metabolic activation system in cultured hamster cells[1]

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Summary. The effect of carcinogenic polycyclic hydrocarbons on the chromosomes of cultured Chinese hamster cells was investigated. Contrary to earlier reports it was observed that benz(a)anthracene, benzo(a)pyrene, 7,12-dimethylbenz-(a)anthracene and 3-methylcholanthrene were effective in causing chromosomal aberrations without any exogenous metabolic activation. Duration of incubation with these agents may be the cause of difference in results. Importance of prolonged treatment period is discussed.

Investigating the effect of chemicals in causing chromosomal aberrations is a useful tool for rapid screening of mutagens and carcinogens in the environment. Recent studies on cultured mammalian cells indicate that certain carcinogenic polycyclic hydrocarbons (CPH) require metabolic activation to produce a clastogenic effect[2-4]. The activation system utilized in these studies consisted of rat liver microsomal fraction (S_9 or S_{15}) or irradiated feeder layer of Syrian hamster cells. The present investigation describes the production of chromosomal abnormalities by treatment with polycyclic hydrocarbons without any exogenous activation in cultured Chinese hamster cells.
V_{79} Chinese hamster cells used in this study were routinely cultured in 75 cm^2 Corning plastic tissue culture flasks in 10 ml of Eagle's minimum essential medium (MEM) supplemented with 10% fetal bovine serum and antibiotics (penicillin-G, 100 units/ml and streptomycin 100 µg/ml). The cells were grown at 37 °C in an atmosphere of 95% air and 5% CO_2. To test the effects of CPH about 2×10^5 cells were plated in 25 cm^2 plastic flasks in 5 ml of growth medium. After cell attachment the cultures were exposed to test compound dissolved in acetone and suspended in culture medium for 24 h. The CPH namely benz(a)anthracene (BaA), benzo(a)pyrene (BaP), 7,12-dimethylbenz-(a)anthracene (DMBA), and 3-methylcholanthrene (MC) were obtained from Eastman Kodak Co., Rochester, N.Y. The concentrations of the compounds used were 0.6, 1.25, 2.5 and 5.0 µg/ml. The control cultures contained 0.1% acetone and were incubated under identical conditions.
For chromosome analysis, the cells were harvested immediately after the end of treatment period. 4 h prior to harvest, colcemid (0.1 µg/ml) was added to each flask. The growth medium was then discarded, the cultures washed, trypsinized, centrifuged (800–1000 rpm, 10 min) and the supernatant aspirated. The pellet was suspended in 1 ml of 0.075 M KCl (37 °C) for 10 min; an equal amount of fixative (1 part glacial acetic acid : 3 parts methanol) added to it and centrifuged (800–1000 rpm, 10 min). The cells were then washed in fixative; centrifuged and resuspended in 1 ml of fresh fixative for 10 min. The concentrated cell suspension was dropped on chilled wet slides and allowed to dry overnight. The slides were stained with 2% Giemsa solution. The type and number of chromosomal abnormalities were assessed by examining 100 well spread metaphase plates from each treatment. The experiments were repeated twice.
The results of clastogenic effects induced by polycyclic hydrocarbons on V_{79} Chinese hamster cells are summarized in the table. It was noticed that chromosomal gaps were the most common abnormality 24 h after treatment with these compounds, accounting for approximately 25–50% of the induced aberrations. The interpretation of gaps has been a matter of controversy. In present study the gaps were considered to be small, discrete, achromatic bands on one or both chromatids. Most of the gaps were confined to one of the sister chromatids that make up the chromosome arm but occasionally they were found on both chromatids at the same location on the arm. Chromosomal gaps were observed in each concentration of hydrocarbons used as well as the controls, however, the frequency was much more in the former. For instance, only 3% of the control cells showed such gaps compared to 27% of the cells treated with 5 µg/ml of BaP. Chromosome rings, while not that common were seen in metaphase which also showed small, diffuse, double chromatin elements interpreted as acentric fragments. These acentric fragments are considered to be