brain levels of [^{3}H]α-MeDA occurred at approximately the same time as the non-amphetamine-like behavior (greatly reduced response to external stimuli, decreased motor activity, and signs of fatigue 6–18 hr after MDA administration to mice[1, *]) supports the hypothesis that α-MeDA may be the mediator.

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Effect of 1-(*p*-methoxybenzyl)-6,7-methylenedioxyisoquinoline on mitochondrial respiration

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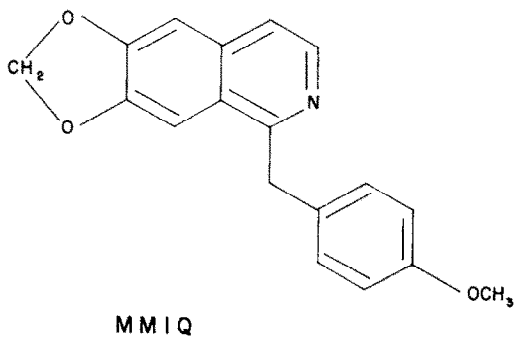
The isolation of an alkaloid extracted from a species of *Ocotea* (*lauracea*) which grows in the Amazon region, Brazil, has recently been reported. Its chemical structure is 1-(*p*-methoxybenzyl)-6,7-methylenedioxyisoquinoline (MMIQ) (Fig. 1)[1]. The compound inhibits the conversion of cyclic 3′,5-AMP to 5′-AMP[1] and influences the contraction–relaxation cycle of smooth muscle. MMIQ is hydrophobic and possesses a positive charge at a pH around 7. Since certain hydrophobic compounds with a positive charge inhibit mitochondrial oxidative phosphorylation[2–4], the effect of MMIQ on the respiration and phosphorylation processes of mitochondria was explored. The results show that MMIQ inhibits the electron-transfer process of mitochondria.

Rat liver mitochondria were isolated in 0.25 M mannitol, 2 mM Hepes and 0.1% bovine serum albumin (pH 7.4). EDTA submitochondrial particles were prepared as described elsewhere[3]. Oxygen uptake was measured either with a Gilson or a YSI apparatus adapted with a Clark electrode. The ATPase activity of submitochondrial particles was measured in a mixture that contained 0.24 M mannitol, 10 mM Tris–HCl (pH 7.4), 11 mM ATP and 4 mM $MgCl_2$ in a final volume of 1.0 ml. The incubation time was 10 min at a temperature of 30°. The reaction was stopped with 6% trichloroacetic acid (final concentration). Inorganic phosphate was determined in the supernatant



MMIQ

Fig. 1. Chemical structure of MMIQ.

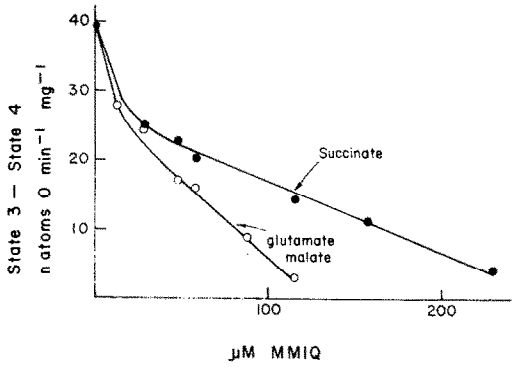


Fig. 2. Effect of MMIQ on the State 3–State 4 respiration of rat liver mitochondria. The incubation mixture contained, in a final volume of 2.0 ml, 10 mM glutamate, 10 mM malate, 5 mM phosphate (pH 7.4), 0.25 M mannitol, 1 mg of bovine serum albumin, 1.5 mg of mitochondrial protein and the indicated concentrations of MMIQ. State 3 was induced by the addition of 1 μmole ADP. Where indicated the mixture contained 10 mM succinate and 3 μg rotenone instead of glutamate-malate. Temperature was 27°. The results show enhancement of the respiratory rate attained by the addition of ADP to mitochondria incubated with the indicated concentration of MMIQ. For the effect of MMIQ on State 4, see text.

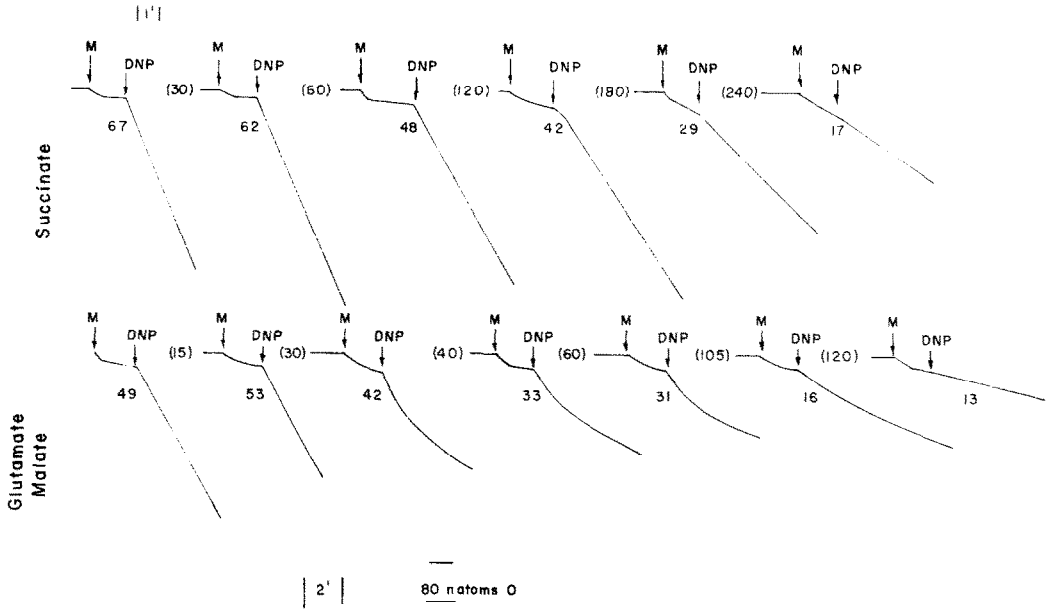


Fig. 3. Effect of MMIQ on uncoupler stimulated respiration. The incubation conditions were as in Fig. 2, except that 10^{-4} M DNP was added where shown. The numbers in parentheses indicate the μM concentration of MMIQ, while the numbers to the left of each trace show the rate of respiration (natoms O min^{-1} mg^{-1}) after the addition of DNP.

fraction according to Sumner [5]. MMIQ was added as an ethanolic solution; the amount of ethanol added did not modify the parameters studied (10 μl/ml of incubation mixture was the highest concentration of ethanol employed). At the highest concentration employed, MMIQ did not precipitate in the respective incubation mixtures. Each experiment was repeated at least three times with different mitochondrial preparations, and the results showed a variation of less than 10 per cent.

The experiments in which the effect of MMIQ on State 3 and State 4 respiration of mitochondria was assayed showed that State 3 respiration with glutamate-malate as substrate was strongly inhibited by MMIQ (Fig. 2), half-maximal inhibition being attained by approximately 30–40 μM MMIQ. State 4 respiration was not affected by MMIQ (data not shown).

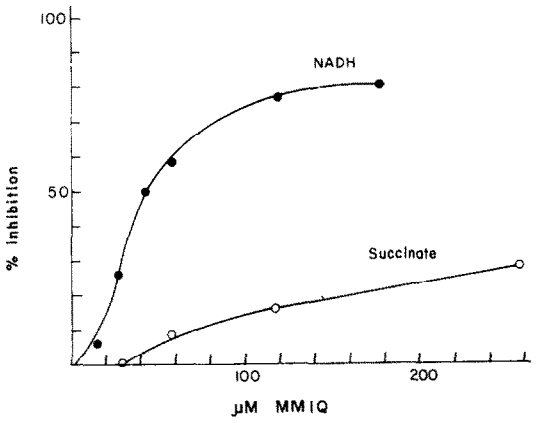


Fig. 4. Effect of MMIQ on the respiration of submitochondrial particles. The incubation mixture contained 0.25 M mannitol, 20 mM Tris–HCl (pH 7.4), and 1 μmole NADH or 10 mM succinate. The particles (0.8 mg protein) were incubated for 2 min with the indicated concentrations of MMIQ before the addition of substrate. The results express the per cent inhibition of respiration attained by the indicated concentrations of MMIQ. In the experiment shown, respiration in the absence of MMIQ was 354 and 280 natoms O min^{-1} mg^{-1} with NADH and succinate as substrate respectively.

With succinate (plus rotenone) as substrate (Fig. 2), State 3 respiration was also inhibited by MMIQ, but higher concentrations of the drug were required to induce an inhibition of State 3 respiratory rates equivalent to that attained with glutamate-malate as substrate. With succinate as substrate, concentrations of MMIQ above 100 μM increased State 4 respiratory rate from a rate in the control of 10 natoms O min^{-1} mg^{-1} to about 16 natoms O min^{-1} mg^{-1}.

The effect of MMIQ on respiration stimulated by 2,4-dinitrophenol (DNP) was examined (Fig. 3). With glutamate-malate as substrate, MMIQ also inhibited the DNP-stimulated oxygen uptake. However it should be noted that, within a certain concentration range of MMIQ (30–105 μM), DNP induces an enhancement of respiration that is not linear with time; that is, after an initial increase in the respiratory rate, the DNP-stimulated oxygen uptake starts to decline until it reaches the rates shown in Fig. 3. With succinate as substrate, MMIQ diminished the rate of the DNP-stimulated respiration, but at concentrations significantly higher than those that decreased the respiration with glutamate-malate.

The MMIQ-induced inhibition of electron transport in intact mitochondria could be due to inhibition of influx of substrates into the mitochondria or to an effect on the electron transport chain. Thus, its action on the respiration of submitochondrial particles in which permeability barriers do not exist was studied (Fig. 4). MMIQ inhibited by approximately 80 per cent the aerobic oxidation of NADH at concentrations which affected, although slightly, the aerobic oxidation of succinate.

MMIQ is an inhibitor of electron transfer in mitochondria. Its main action is on the NADH-CoQ segment of the respiratory chain. At higher concentrations it also affects the oxidation of succinate. It is of importance to point out that MMIQ does not affect the ATPase activity of submitochondrial particles (0.8 μmoles ATP hydrolyzed min^{-1} mg^{-1} under the conditions outlined above); thus, the

action of MMIQ would seem to be limited to the respiratory chain. However, if the data in Figs. 2 and 3 are compared to the results shown in Fig. 4, it may be observed that MMIQ inhibits succinate oxidation in mitochondria but not in submitochondrial particles. Therefore, the possibility that MMIQ affects the influx of succinate into the mitochondria cannot be fully discarded.

In comparison to other inhibitors of electron transport which preferentially act on the NADH-CoQ segment of the respiratory chain, MMIQ possesses some unique properties. It differs from rotenone and piericidin [6, 7] in that these two inhibitors are more potent than MMIQ [8], and in addition, MMIQ inhibits the oxidation of succinate by intact mitochondria. In this respect, it is more like octylguanidine [2, 3], which at low concentrations (30 μM) inhibits the coupled oxidation of NAD-dependent substrates and at much higher concentrations (300 μM) inhibits the coupled oxidation of succinate. Nevertheless, the inhibiting action of octylguanidine is reversed by uncouplers [9], while MMIQ inhibits uncoupler-stimulated respiration. Since MMIQ, octylguanidine and ethidium bromide possess a positive charge at a pH around 7, these three compounds most probably act on the same site or through a similar mechanism; however, it is probable that the binding of MMIQ to the membrane would be more stable owing to stronger hydrophobic interactions in addition to charge interactions. Thus, MMIQ would not be released from the membrane by the uncoupler-induced collapse of the electrochemical potential, as is the case for octylguanidine and ethidium bromide [2, 4].

Another important characteristic of MMIQ is that it inhibits electron transport at concentrations 50 per cent lower than those that inhibit phosphodiesterase activity [1]. This may indicate that MMIQ affects the contraction–relaxation cycle of muscle (see Ref. 1) by interfering with the mitochondrial energy-transducing system, particularly if it is considered that mitochondria, by regulating cytoplasmic Ca^{2+} levels, may affect muscle contraction [10]. This is a possibility that will be explored.

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Elevation of brain histamine levels by diaminopyrimidine inhibitors of histamine *N*-methyl transferase

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In the central nervous system (CNS) of mammalian species, histamine is metabolized primarily by methylation of the imidazole ring [1]. This reaction requires *S*-adenosyl methionine (SAM) as the methyl donor and is catalyzed by the enzyme histamine *N*-methyl transferase (HMT). In rats, however, tissues other than brain contain histaminase and thus have an alternative pathway for the metabolism of histamine. Among several antimalarial drugs that inhibit the methylation of histamine *in vitro*, pyrimethamine, a 2,4-diaminopyrimidine, has been reported to be a potent inhibitor of this enzyme [2]. The initial clinical studies of a pyrimethamine analog, metoprine, related to its evaluation as an anticancer agent, indicated that this compound produced CNS, cutaneous, and gastrointestinal toxicities [3–5], possibly involving inhibition of histamine catabolism. Similar CNS and cutaneous toxicities occurred during clinical studies of the anticancer agent triazinate, a diamino-*s*-triazine [6]. This report describes the effects of these anticancer agents on the activity of HMT *in vitro* and *in vivo*.

[^{14}C]methyl-SAM (sp. act. 57.8 mCi/m-mole) and [^{3}H]methyl-SAM (sp. act. 10.5 Ci/m-mole) were purchased from New England Nuclear; histamine, quinacrine and chloroquine were secured from Sigma Chemical Co., St. Louis, MO. Triazinate was obtained from the Drug Development Branch of the National Cancer Institute, Bethesda, MO. Pyrimethamine [2,4-diamino-5-(4′-chloro phenyl)-6-ethyl pyrimidine; BW 63U], metoprine [2,4-diamino-5-(3′,4′-dichlorophenyl)-6-methyl pyrimidine; DDMP; BW 197U], etoprine, the corresponding 6-ethyl analog of metoprine (DDEP; BW 276U) and triazinate [α-2-chloro 4-[4,6-diamino-2,2-dimethyl-*s*-triazine-1(2H)-yl]phenoxyl]-*N*,*N*-dimethyl-*m*-toluamide ethanesulfonic acid; TZT; NSC 139105] were dissolved in 0.01 N HCl prior to use.

HMT was partially purified from the cerebral cortex of bovine brain, using the ammonium sulfate fractionation and dialysis steps described by Brown *et al.* [7]. HMT incubation mixtures consisted of 10 μmoles of sodium phosphate buffer, pH 7.4, 15 nmoles histamine, 14.2 nmoles [^{14}C]-SAM, enzyme and, where appropriate, inhibitor, in a total volume of 300 μl. The reaction was initiated by the addition of [^{14}C]-SAM and incubated for 15 min at 37°. The reaction was terminated by the addition of 0.5