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G.A. Yacout
Biochemistry Department,
Faculty of Science,
Alexandria University,
Alexandria, Egypt

E-mail: galila_69@yahoo.com

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Biochemical Studies on Rabbits Treated With Novel Pyridine and Dihydropyridine Derivatives and Vitamin E

G.A. Yacout

The present study aims to compare the effects of two compounds 4-(2-phenyl-1,2,3-triazole)-3, 5-ethoxycarbonyl-2, 6-dimethyl-1,4-dihydropyridine (DHP) and 4-(2-phenyl-1,2,3-triazole)-3,5-diethoxycarbonyl-2,6-dimethylpyridine (PY) on rabbits. Both found to be good activators of MAO-B. The activation using PY was increased by combination with vitamin E. Also, the effects of DHP and PY on rabbits including liver RNA and DNA content as well as serum clinical analysis were investigated. The obtained results showed a decrease in RNA content while showed an increase in the DNA content to the level of 68 and 124%, respectively.

Key words: Pyridine derivatives, vitamin E, MAO-B, activators

INTRODUCTION

Monoamine oxidase is an outer mitochondrial membrane protein that catalyses the deamination of a number of neurotransmitters and dietary amines. Interestingly, MAO activity was determined in schizophrenic patients and showed a tendency to express a low activity^[1]. Extensive studies have led to new class of potent and selective reversible MAO inhibitors including, oxadiazole derivatives^[2] and substituted oxadiazole derivatives^[3]. On the other hand, some pyrrole derivatives containing pyridyl group were found to be powerful activators^[4]. Also, it was reported that 4 (or 5) diazoimidazole-5 (or 4)-carboxamide was an activator to MAO^[5]. In addition, previous data showed that the enzymatic activity of MAO-B was increased by aluminum^[6], by ethanol and phenylamine^[7] or by thalium acetate^[8].

Furthermore, many investigations revealed that pyridine or dihydropyridine derivatives have numerous biological activities such as inhibition of HMGCoA-reductase^[9], antihypertensive prodrug^[10], K⁺ channel openers^[11], c-AMP phosphodiesterase human platelet aggregation inhibitors^[12], antiulcer agents^[13,14], antimycobacterial activity^[15] and antimalarial activity^[16]. Also, some dihydropyridine derivatives act as highly selective adenosine receptor antagonists^[17] as well as antiallergy agents^[18].

On the other hand, vitamin E was found to play an important role in the disturbance of monoamine metabolism in rat brain^[19]. Therefore, it was of interest to study the effect of the two novel pyridine and dihydropyridine derivatives, Fig. 1 on the activity of brain

MAO-B and investigate the range of alteration due to the presence of vitamin E, looking forward to use these compounds in the treatment of some diseases related to MAO activity.

MATERIALS AND METHODS

Animals: Fifteen healthy rabbits weighing 1000±100 g each, were divided into five groups. The first group N served as control received normal diet and 1 ml of corn oil daily. The second group received normal diet with a daily oral dose of DHP in corn oil (10 mg ml⁻¹), while the third group was given normal diet with daily oral dose of PY in corn oil (10 mg ml⁻¹). The fourth group received normal diet with a daily dose of vitamin E (10 mg ml⁻¹ corn oil). The fifth group was given normal diet with daily dose of vitamin E (10 mg ml⁻¹), then after two hours, received 10 mg ml⁻¹ of PY. The experiment was carried out for seven days, after those animals were scarified. The brain was removed and analyzed for MAO activity, while the removed liver was analyzed for DNA and RNA content. In addition, blood samples were collected separately and the obtained sera were ready for analysis.

Chemicals: The chemicals used for buffer, orcinol reagents, diphenylamine reagents, benzyleamine, perchloric acid and bovine serum albumin were of the highest quality available. Vitamin E was obtained from ICN Biochemicals inc. Aurora, Ohio. RNA from *E.coli* and DNA sodium salt from calf thymus were purchased from BDH Chemicals Ltd. (Poole, England). DHP and PY were kindly synthesized and purified by M.El-Sadek and his coworkers, department of chemistry, Faculty of Science, Alexandria, EGYPT.

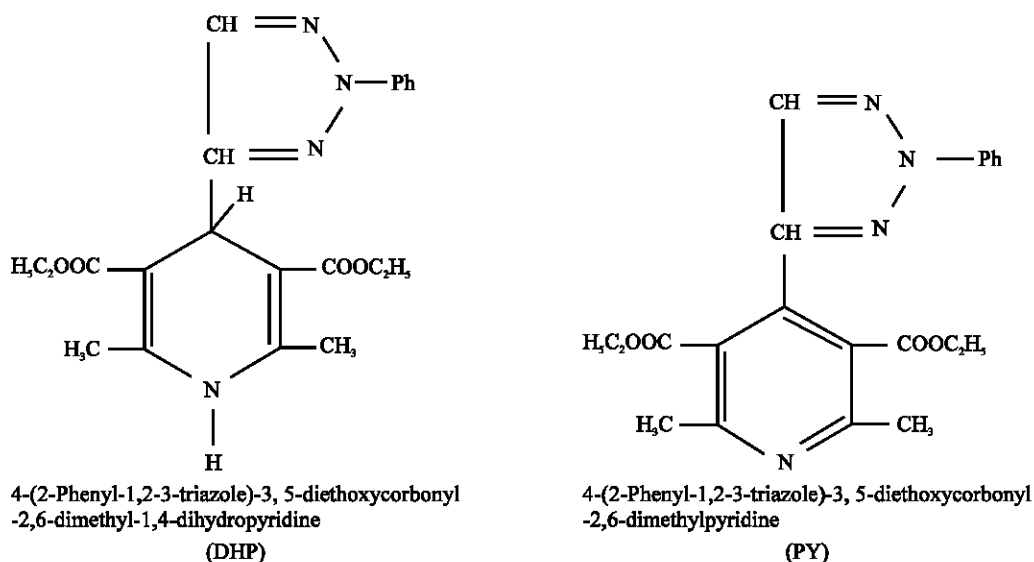


Fig. 1: Examined compounds

Serum clinical analysis: Each obtained serum was assayed for aspartate aminotransferase/alanine aminotransferase (AST/ALT), albumin, bilirubin, urea/creatinine, glucose, triglycerides and serum creatine kinase (SCK) using kits produced by Boehringer Mannheim, Germany.

Tissue and enzyme preparations: Rabbit brain (7.3 g) was homogenized following the procedure^[20], in 9 volumes of ice-cold 0.1 M sodium phosphate buffer (pH 7.4), using Tekmar Tisumizer. The homogenate was centrifuged for 10 min at 600 g, take supernatant I and the residue was homogenized with 0.3 M sucrose solution then centrifuged again, giving supernatant II, then collected the two supernatants. After that, brain mitochondria were obtained by completes the differential centrifugation using cooling centrifuge, Hettich Zentrifugen EBA 12 R. The sediment mitochondria were suspended in phosphate buffer to give final volume of 1 ml g⁻¹ wet weight of brain tissue. All preparations of all samples were stored at -15°C until required. These preparations were diluted about 10-fold with buffer before use.

Assay of MAO-B activity: MAO activity of each obtained mitochondrial fraction was assayed with benzylamine as substrate (10 mM), by continuous recording of the increase in extinction at 250 nm, that produced at 30°C, for 15 min and pH 7.4 when 0.1 ml benzylamine was added to 0.2 ml enzyme preparation and 2.9 ml of 0.1M sodium phosphate buffer, using Ultrospec 1000 spectrophotometer, Pharmacia Biotec, that according to the method Gorkin *et al.*^[21]. Blank experiment was prepared without benzylamine.

Estimation of protein content: The protein content of each enzyme preparation was estimated according to Lowry *et al.*^[22], using bovine serum albumin as standard.

Fractionation of liver homogenate for DNA and RNA: 0.5 g from the liver of DHP-treated rabbit and PY-treated ones as well as control N was homogenized in 5 ml of 0.25 M sucrose solution, separately. Each obtained homogenate was fractionated for RNA and DNA as previously described by Keleti and Lederer^[23], giving fraction I for RNA and fraction II for DNA measurements.

Estimation of hepatic RNA and DNA: Each obtained fraction I (0.5 ml) was mixed with 1.5 ml of orcinol reagent according to Keleti and Lederer^[24], the developed color was measured at 665 nm, using Photomech 301-D⁺ spectrophotometer. Blank was carried out with 0.5 ml of 0.3 M KOH-0.5 M perchloric acid mixture (3:5, v/v) and then treated as test sample. Fraction II (0.9 ml) was

mixed with 1.8 ml diphenylamine reagent, the developed color was measured at 596 nm against blank solution, containing 0.9 ml of 0.5 M perchloric acid and treated as test samples according to Keleti and Lederer^[25]. Standard curves of RNA and DNA were used.

The work was carried out in the department of Biochemistry, Faculty of Science, Alexandria University, EGYPT.

RESULTS

In the present study, MAO-B was prepared from the brain of DHP, PY, vitamin E, E+PY-treated rabbits and control N, each separately. The specific activity was measured in each case using benzylamine substrate, Table 1. All the examined compounds were found to be activators to MAO, with specific activity, 7.55×10^{-5} , 5.61×10^{-5} , 6.13×10^{-5} and 7.40×10^{-5} compared to control 0.65×10^{-5} $\mu\text{M min}^{-1} \text{mg}^{-1}$. Table 2 showed that DNA content in DHP and PY-treated groups were increased to be 0.42 and 0.56 compared to control 0.25 mg g⁻¹ with an increased values to about 68 and 124%, respectively. Meanwhile, estimation of RNA content showed a slight decrease to about 8.68 and 10.22 compared to control 10.56 mg g⁻¹. Table 3, demonstrates liver, kidney and heart muscle function-parameters. The obtained results revealed that AST/ALT, ratio was 0.67, 1.29 and 0.95 compared to control 0.74, respectively. Albumin level was 3.81, 3.75 and 3.93 compared to control 3.93 g dL⁻¹,

Table 1: Specific activity of MAO-B of DHP, PY, Vitamin E and E+PY-treated rabbits compared to control N at substrate concentration (10 mM)

Sample	Total activity ($\mu\text{M min}^{-1}$) $\times 10^{-5}$	Total protein (mg)	Specific activity ($\mu\text{M min}^{-1} \text{mg}^{-1}$) $\times 10^{-5}$
N	0.51	0.78	0.65
DHP	3.93	0.52	7.55
PY	2.81	0.50	5.61
E	4.11	0.67	6.13
E+PY	5.11	0.69	7.40

Table 2: The mean values of DNA and RNA content in liver cells of DHP and PY-treated rabbits compared to control N

Sample	DNA (mg g ⁻¹)	RNA (mg g ⁻¹)
N	0.25	10.56
DHP	0.42	8.68
PY	0.56	10.22

Table 3: The mean levels of serum parameters of DHP, PY and E+PY-treated rabbits compared to control N

parameter	N	DHP	PY	E+PY
AST/ALT	0.74	0.67	1.29	0.95
Albumin g dL ⁻¹	3.93	3.81	3.57	3.93
Bilirubin mg dL ⁻¹	0.37	0.40	0.20	0.50
Urea/creatinine	82.80	79.50	80.00	70.00
Glucose mg dL ⁻¹	119.00	123.00	121.00	119.00
Triglycerides mg dL ⁻¹	40.40	43.00	55.55	68.60
SCK U/L	1636.00	1565.00	1722.00	1542.00

respectively. Also, the level of bilirubin was found to be 0.4, 0.2 and 0.5 compared to control 0.37 mg dL⁻¹. Estimation of urea/creatinine ratio showed a slight decrease, 79.52, 80.0 and 70.0 compared to control 8.28, respectively. The level of triglycerides of the examined rabbits was increased 43.0, 55.55 and 68.6 compared to control 40.4 mg dL⁻¹, while glucose level seems to be slightly changed, 123, 121 and 119 compared to control 119 mg dL⁻¹. SCK was found to be 1565, 1722 and 1542 compared to control 1636 UL⁻¹, respectively.

DISCUSSION

In recent years, sufficient evidences has surfaced to implicate low molecular weight organic compounds in certain neurological disorders such as 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine which considered as a compound capable of inducing condition most similar to parkinsonism^[26].

It was appeared that brain as an important target organ to the alteration in MAO-B activity through the treatment with DHP, PY, vitamin E as well as E+PY. MAO-B activity was increased by more than 11.52-fold and 8.56-fold in case of DHP and PY-treated rabbits respectively, that means DHP or PY may have a tendency towards activation of MAO-B suggesting that, there was a binding between each examined compound and the specific activator site on the enzyme facilitates the enzyme binding to substrate and enhancing the rate of interaction. Furthermore, a considerable relationship was observed between the structure of DHP or PY and their effect on MAO in which DHP was better activator than PY, may be depends on the presence of the removable protons in the first compound. Meanwhile, the combined administration of vitamin E before the treatment with PY could improve the binding of PY with the activator site of the enzyme by more than 11.29-fold, is very beneficial activator for MAO. Despret *et al.*^[27] suggested that desaturase enzyme activity was increased when vitamin E increased in the brain. Also, the obtained results revealed that MOA-B activity was increased by more than 9.35-fold when the content of vitamin E in the diet was increased. That probably due to the presence of vitamin E as membrane stabilizing agent, that protect the cell membrane against lipid peroxidation which may could be happened in various rabbit brain fractions, that was in agreement with the obtained data^[28].

On the other hand, study of the hepatic DNA content, revealed that DHP and PY have powerful positive effects on the replication of DNA. However, a significant lowering in RNA content of PY-treated rabbit was

observed greater than that PHD-treated ones compared to control. the obtained lowered value probably due to the short-term experiment oral administration of the tested compounds.

Study of serum clinical parameters of DHP-treated rabbit as will as E+PY groups showed that AST/ALT ratio in the first and second cases were slightly changed, since the normal ratio was between 0.7-01.4. Also, the levels of albumin in the treated rabbits were found to be near the control. Meanwhile serum bilirubin decreased in PY-treated rabbits while increased in case of E+PY-treated ones. That indicates the slight hepatotoxic effect of the examined compounds^[29]. Also, group E+PY was found to be within normal range better than PY alone, since vitamin E afforded protection against liver damage. That was in agreement with Kouronakis *et al.*^[30]. Furthermore, estimation of urea/creatinine ratio in each treated group, compared to control was found to be within range, indicates the normal renal function. In addition, there was an increase in the levels of glucose and triglycerides in case of DHP and PY-treated rabbits, while E+PY group showed a normal level of glucose associated with increased level of triglycerides, probably due to the pretreatment with vitamin E. That was in agreement with the data of Gorbunov *et al.*^[31] who study the action of vitamin E on adenylate cyclase system functioning on the lipid bilayer micro viscosity of the rat brain cells. He was found that, the preincubation of cells with alpha-tocopherol will decrease the adenylate cyclase activity to the normal level. It was appeared due to the modification of the lipid-protein interaction of annular lipids with activated complex of catalytic subunit guanyl nucleotide-binding protein. That affects on the phosphorylation of phosphorylase and lipase enzymes, leads to a decrease in glycogenolysis, so the blood glucose level will be normal, while increase in triglycerides level, probably due to decrease in the lipolysis. Estimation of SCK activity of DHP or PY-treated rabbits showed an alteration, which may be prevented by the pretreatment with vitamin E, that affords a protection against muscular dystrophy^[32].

In conclusion, this study showed that DHP or E+PY is powerful activator for MAO-B, as well as inducer for DNA replication with low degree of toxicity.

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