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INTERACTIONS OF L-DOPA/PARGYLINE, AN ORGANOPHOSPHORUS COMPOUND (VX) AND ITS ANTIDOTES IN RATS:NEUROCHEMICAL AND TOXICOLOGICAL ASPECTS

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In the neurochemical experiments rats were pretreated with L-DOPA and pargyline, in order to enhance the concentration of dopamine and noradrenaline in the central nervous system. One hour later, they were treated with 0.5 LD50 O-ethyl-S-(diisopropylaminoethyl)-methyl-phosphonothioate (VX) and 60 minutes thereafter with atropine, 2-hydroxyiminomethyl-pyridinium-1-methyl-4'-carbamoyl-pyridinium-1'-maethylether dichloride HI-6 and diazepam. The caudate nucleus monoamine content was estimated 3 hours after the administration of VX. L-DOPA/pargyline pretreatment resulted in significant increases in the concentrations of both the monoamines studied, while VX per se had a qualitatively simular, but quantitatively minor though significant influence as well. The antidotes caused no neurochemical alterations, when given alone. When injected after the catecholamine concentrations had previously been increased, VX produced a moderate decrease in their levels, which was further accentuated following administration of the antidotes, but not enough to normalize their values. In the toxicological experiments, where antidotes were given 15 minutes after VX, L-DOPA/pargyline pretreatment enhanced the acute toxicity of VX and insignificantly diminished the protective efficacy of the antidotes employed.

Key words: rat, brain catecholamines, organophosphours compouds, VX, acute toxicity, acetylcholinesterase.

INTRODUCTION

Organophosphorus(OP) compounds exert their poisonous effects predominatly through inhibition of acetylcholinesterase (AChE) in all the vital organs and tissues. The main result is accumulation of excessive quantities of acetylcholine (ACh) in the cholinergic synapses, which leads to receptor overstimulaton and the occurence of typical cholinergic signs and syptoms in the intoxicated individual (Namba et al., 1971).

However, some of the symptoms observed, i. e. hypothermia, some behavioural changes etc., cannot be directly atributed to the mechanism described, because they can be reproduced by pharmacological manipulations, which lead to alteration in the brain level of some biogenic amines (Coudray-Lucas et al., 1981; Fernando et al., 1984a,1984b). Confirmation of the hypothesis that the abovementioned phenomena are of monoaminergic origin were some experimental findings in animals exposed to sublethal doses od OP AChE inhibitors, in which changes in the brain monoamine concentrations occurred after systemic (Coudary-Lucas et al., 1987), as well as after local, i. e. intrastriatal application of OP compounds (Robinson et al., 1986;Robinson and Hambrecht, 1988).

Some previous investigations carried out in our laboratory were conducted to elucidate the correlation between the neurochemical and toxicological consequences of OP poisoning. Since only central monoaminergic store depletion enhances the toxicity of OP compounds and reduces the protective efficacy of standard antidotes (Takahashi et al., 1987; Stojiljković et al., 1989, 1990), the purpose of the present study was to estimate whether a previous increase of brain catecholamine concentrations would affect the acute toxicity of the AChE inhibitors and the therapeutic effectiveness of the standard antidotes, or not.

MATERIALS AND METHODS

Male Wistar rats, weighing 200-250 g were used throughout the experiments. VX (O-ethyl-S-(diisopropylaminoethyl)-methyl-phosphonothioate) was synthesized in our laboratory and used as a product of 99.8% purity. Stock solutions (1% w/v) were made by dilution of VX in 2-propanol and storage at +4° C. Immediately before injecting the rats a fresh solution of the OP was prepared by futher dilution of the stock solution in 0.9% saline. Pargyline hydroshloryde was purchased from Sigma (St. Louis, USA), l-b-(3,4-dihydroxyphenyl)-alanine (L-DOPA) from Kosh-Light laboratories (Cionbrooks Bucks, UK), atropine sulphate monohydrate from Merck (Darmstadt, FRG), diazepam (Apaurin®), from Krka (Novo Mesto, Slovenia), while HI-6 (2-hydroxyiminomethyl-pyridinium-1-methyl-4'-carbamoyl-pyridinium-1'-methyleth er dichloride monohydrate) was synthesized by Bosnalijek (Sarajevo, Bosna and Hercegovina).

The animals were injected subcutaneously (s. c.)with 0.5 median lethal dose (LD50) of VX. One hour before that one group of them had been pretreated by an intraperitoneal (i. p.) injection of L-DOPA and pargyline, 200 and 25 mg/kg of body weight, respectively. The other group received the same volume of saline instead. One hour after receiving VX, the rats were treated intramuscular (i. m.) with either atropine/HI-6/diazepam (10, 37.7 and 2.5 mg/kg, respectively) or saline. There hours folowing intoxication all the rats were sacrificed by decapitation, their caudate nuclei dissected and used for catecholamine level determination which was performed fluorometrically (Laverty and Taylor, 1968). The pretreatment and treatment conditions for the acute toxicity study

were the same as those previously described, except that the doses of VX employed were in the lethal range. Also, for the same reason the time interval between the intoxication and administration of antidotes had to be shortened to only 15 minutes, in order to allow survival. Thus the occurrence of numerous unavoidable deaths of the animals before the application of the antidotes was prevented. All the animals were divided into groups of 8 rats each and left after injection for 24-hours survival rate registration. These data were used for the calculation of LD50 values (Litchfield and Wilcoxon, 1949).

The statistical of the differences between the parametric data was tested by means of Students's t-test and P values less than 0.05 were considered significat.

RESULTS

Neurochemical studies. While VX *per se* moderately, though significantly, increased caudate nucleus dopamine concentration to 16% above the control value, noradrenaline content was to a similar extent decreased by 14%. Antidotes, given alone, did not influence caudate nuclei catecholamine levels. Much more drastic neurochemical alterations were found in the groups of rats with L-DOPA/pargyline. When no other chemical was administered dopamine and noradrenaline concentrations were increased roughly by factors of 2.6 and 1.4,respectively.

Administration of VX produced diverse changes in the previously elevated contents of dopamine and noradrenaline. This the later remained practically unchanged, the former was significantly reduced from 259% to 212%, as compared with the control value. However, the dopamine content still remained double. Although the antidotes completely normalized the VX-induced neurochemical alterations found in saline-pretreated animals, when the rats were also pretreated with L-DOPA/pargyline caudate nucleus dopamine content remained signifincantly increased above the control level, though the enhoncement above the control value was halved from + 112% to + 71%. Noradrenaline concentration, after the application of the antidotes, was decreased from + 146% to + 124% of the control, which was, nonetheless, enough to be insignificantly different from the control noradrenaline level (Table 1).

Toxicological studies. L-DOPA/pargyline pretreatment decreased the LD50 value of VX by one third, which was shown not to be statiscally significant. The efficacy of the antidotal protection remained unchanged both in pretreated and in practically unpretreated animals, with protective ratios of 2.9 and 3.3, respectively (Table 2).

DISCUSSION

Pretreatment of the rats with L-DOPA, a dopamine precursor, and pargyline, a monoamine oxidase inhibitor, results in a significant increase of caudate nucleus catecholamine content, which is significantly reduced by giving VX, and futher diminshed after administration of the standard antidotes. This means that both the OP compound and its antidotes exert similar, instead of antagonis-

tic effects upon dopamine and noradrenaline levels. In the acute toxicity study, however, the antidotes cleary produced the same significant protective effect both in saline - and in L-DOPA/pargyline-pretreated animals intoxicated with VX.

Table 1. Influence of L-DOPA/pargyline, VX, its antidotes and their combinations on catecholmine steady-state levels in rat caudate nucleus

Treatment (mg/kg)	Dopamine (% of control ± S.E.M., n = 6)	Noradrenaline (% of control ± S.E.M., n = 6)
L-DOPA (200) pargyline (25)	259 ± 4[a,c]	142 ± 4[a]
VX (0.5 LD_{50})	116 ± 3[a,b,c]	86 ± 3[a,b,c]
atropine (10) HI-6 (37.7) diazepam (2.5)	99 ± 3[b,c]	96 ± 5[b,c]
L-DOPA (200) pargyline (25) VX (0.5 LD_{50})	212 ± 7[a,b]	146 ± 4[a]
VX (0.5 LD_{50}) atropine (10) HI-6 (37.7) diazepam (2.5)	91 ± 6[b,c]	89 ± 7[b,c]
L-DOPA (200) pargyline (25) VX (0.5 LD_{50}) atropine (10) HI-6 (37.7) diazepam (2.5)	171 ± 3[a,b,c]	124 ± 9

$p < 0.05$, as compared with a — control, b — L-DOPA/pargyline, c — L-DOPA/pargyline+VX. Control values: dopamine 5870 ± 275 ng/g noraddrenaline 790 ± 36 ng/g.

Table 2. Influence of L-DOPA/pargyline pretreatment on acute toxicity of VX and standard antidote protective efficacy

Pretreatment (mg/kg i.p.)	Treatment (mg/kg i.p.)	LD_{50} (95%-confidence limits (μg/kg s.c.)	Proctective ratio
saline	saline	14.0 (10. 3- 19. 0)	1. 0[b]
saline	atropine (10) HI- (37.7) diazepam (2.5)	46.6 (39. 3- 55. 3)	3.3[a]
L-DOPA (200) pargyline (25)	saline	8.8 (5.8 - 13. 2)	0.6[b]
L-DOPA (200) pargyline (25)	atropine (10) HI- (37.7) diazepam (2.5)	40.2 (39. 4-41. 0)	2.9[a]

[a,b] = $p < 0.05$, as compared with: a — saline + saline, b — saline + antidotes

There are two other studies undertaken with a similar aim. In the first one rabbits were preterated with L-DOPA and, instead of pargyline, with another monoamine oxidase inhibitor JB835 and thereafter intoxicated with diisopropylfluorphosphate (DFP) (Glisson et al., 1974). In these animals DFP caused, country to the results of the present study, a further increase of the dopamine level, and, simillary to our results, a decrease of the noradrenaline concentration in the rabbit brain regions. The second study, carried out in mice, gave results quite opposite to ours (Buccafusco et al., 1988). Reserpine did not influence soman acute toxicity, while our recent resarch indicated that this alkaloid enhanced it (Stojiljković et al., 1989). Further, pargyline pretreatment provided some degree of protection both from behavioural and lethal effects of soman in mice, while in the present investigation L-DOPA/pargyline preteatment exerted almost the opposite effect. These discrepanicies should be understood as the consequencies of the different OP agents (DEP and soman), routs of administration (systemic and local), brain regions and species (mice, rabbit and rats) used. Moreover, it is very hard to generalized of these studies, since it is at the moment almost impossible testimate a clear correlation between the brain monoamine level lateration and the acute toxicity of the subsequently administered cholinesterase inhibitors. This phenomenon could be explained, though still hypothetically, through the predominantly cholinergic mechanism of organophosphate toxic and lethal effects, which are weakly, though sometimes significantly, affected by monoamine changes in the central nervous system (Buccafusco and Aronstam, 1987; Buccafusco et al., 1988). The findings that atropine and other anticholinergic antidotes efficiently antagonize all the toxic effects of the cholinesterase inhibitors (Andén and Wachtel, 1977; Morgan and Pfeil 1979; Tanasijević et al., 1989) are indirect evidence for this. Moreover clonidine, an alpha-andrenergic agonist, reduces cholinesterse inhibitor-induced lethality rates, which is the consequence of inhibition of brain acetycholine liberation (Buccafusco and Aronstam, 1987; Buccafusco et al., 1988). Nevertheless, a possibility that the OP compounds affect the release of catecholamines directly via protein sites that are important in the regulation of that process can not be excluded (Kant et al., 1984).

The neurochemical changes which follow the organophosphate intoxication, among which the most frequently reported are the reduction of noradrenaline (Fosbraey et al., 1990) and the increase of dopamine steady-state content (Stojiljković et al., 1989), are obviously muscarinic in origin and surely not nicotinic. This was demonstrated in rats, where the physostigmine- or oxotremorine-induced noradrenaline depletion could be not be alleviated with mecamylamine, but could be prevented with atropine (Morgan and Pfeil, 1979). Furthermore, acetylcholine accumulated in the central nervous system after cholinesterase was inhibited only during the first 60 minutes following poisoing, which suggests strong autoregulative mechanisms of the brain cholinergic nerons (Robinson and Hambrecht, 1988). Therefore. it is to be expected that the brain monoamine level changes, induced by cholinesterase inhibithors, show neither dose-nor time-dependency, since they reflect the link between the inhibitor used and brain acetylcholine concentration (Ugrešić et al., 1990).

In conclusion, in could be pointed out that the standard antidotes afford good protection in L-DOPA/pargyline pretreated rats both from the neurochemical alternations and from death produced by administration of VX. However, a clear correlation between the previously enhanced catecholamine levels and VX acute toxicity was not revealed, probably due to efficient physiological brain neurotransmitter autocompensatory mechanisms.

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INTERAKCIJA L-DOPA/PARGILINA, VX I NJEGOVIH ANTIDOTA U PACOVA: NEUROHEMIJSKI I TOKSIKOLOŠKI ASPEKTI

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SADRŽAJ

U neurohemijskim eksperimentima pacovi su predtretirani L-DOPA-om i pargilinom, u cilju povećanja koncentracije dopamina i noradrenalina u centralnom nervnom sistemu. Oni su jedan sat kasnije tretirani sa 0.5 LD_{50} otrova VX, a 60 minuta nakon toga atropinom, oksimom HI-6 i diazepamom. Sadržaj monoamina u repatom jedru određivan je tri sata nakon primene VX-a. L-DOPA/pargilinski predtretman rezultovao je značajnim povećavanjem koncentracija oba proučavana monoamina, dok je sam VX imao kvalitativno sličan, ali kvantitativno manji, mada takođe značajni, efekat. Sami antidoti nisu uzrokovali nikakve neurohemijske promene. VX, primenjen nakon što je prethodno koncentracija kateholamina bila povećana, izazvao je umereno smanjenje njihovih nivoa, koje je dalje bilo naglašeno primenom antidota, ali ipak nedovoljno da bi se potpuno normalizovale njihove vrednosti. U toksikološkim eksperimentima, u kojima su antidoti davani 15 minuta nakon VX-a, L-DOPA/pargilinski predtretman povećao je akutnu toksičnost VX-a i neznčajno smanjio zaštitnu delotvornost primenjenih antidota.