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NEUROGLIAL PROTECTION BY DOPAMINE AGONISTS AGAINST LEAD TOXICITY

(With 2 Tables and One Figure)

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أستخدام مقاومات الدوبامين فى حماية الدبق العصبى من التسمم بالرصاص

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لدراسة حماية الدبق العصبى من التسمم بالرصاص بأستخدام مقاومات الدوبامين (ل-دبرانيل، بروموكربتين، برجلويد) تم إ ستخدام الخلايا النجميه العصبية (الأستروسيت) عمرها ١٤ يوما. قسمت مزارع هذه الخلايا الى ثلاثة مجموعات . غذيت المجموعة الأولى على الوسط الغذائى الأساسى دون أى معامله وغذيت المجموعه الثانيه على الوسط الغذائى الاساسى مضافا اليه ١٠ ميكرومول خلات رصاص لمدة ٢٤ ساعة . أما المجموعه الثالثه فقد غذيت على نفس الوسط الغذائى الاساسى وقد تم تقسيمها الى ثلاثة أقسام: (أ) قسم أضيف اليه ١٠ ميكرمول خلات رصاص + ل – دبرانيل ١& ١٠& ١٠٠ ميكرومول لمده ٢٤ ساعه ، (ب) قسم اضيف اليه ١٠ميكرمول خلات رصاص + بروموكربتين ١&١٠& ١٠٠ ميكرومول لمده ٢٤ ساعه ، (ج) قسم اضيف اليه ١٠ ميكرومول خلات رصاص + برجلويد ١&١٠&١٠٠ ميكرومول لمده ٢٤ ساعه. وبدراسة التغيرات المورفولوجيه التى طرأت على الخلايا النجميه العصبيه (مساحه الخلايا – عددها – عدد الزوائد الاوليه والثانويه وكذلك أطوالهما). فقد أظهرت النتائج أن المعامله ب ١٠ ميكرومول خلات رصاص أدت الى ظهور نقص واضح فى التغيرات المورفولوجيه مقارنة بالمجموعه الاولى التى لم تعامل بأى شيئ ٠ بينما ظهر اثر استخدام مقاومات الد وبامين على خلايا الاستروسيت فى المجموعه الثالثه فى حمايتها من إحداث خلات الرصاص لاثرها السمى والضار عليها ٠ وجنبا الى جنب مع التغيرات المورفولوجيه فقد اظهر قياس انزيم لاكتيت دى هيدروجينيز مدى أهميه استخدام مقاومات الدوبامين فى الحفاظ على الخلايا النجميه العصبيه من التاثير السامة لخلات الرصاص مما يسمو بهذا العمل أن يكون محاولة جديده لحمايه الخلايا النجميه العصبيه (الدبق العصبى) من الاثر السام للرصاص.

SUMMARY

Three alternatives for the prevention of lead toxic effects L-deprenyl, bromocriptine and pergolide were analyzed. Primary astrocyte cultures, 14 day in vitro, were exposed to 10 µM lead acetate for 24 hours. This lead

acetate exposed cultures were exposed also to dopamine agonists (L-deprenyl, bromocripine and pergolide in a final concentrations 1,10 and 100 µM for each substance for 24 hours. The morphogical changes of astrocytes (area, number of cells, number of processes either primary and secondary and their lengths) were significantly decreased ($P<0.05$) in lead acetate treated groups in comparison with untreated one. Area and number of astrocytes were increased in cultures treated with both L- deprenyl, bromocriptine and pergolide in all concentrations of these used substances in relation to cultures treated with lead acetate alone. The other morphological changes include number of processes and length of secondary processes were significantly increased than lead acetate treated cultures in almost concentrations used of previousely mentioned dopamine agonists. The cytoplasmic leakage represented in measuring LDH was significantly ($P<0.05$) increased in cultures treated with lead acetate than that untreated, while LDH was significantly ($P<0.001$) reduced in cultures treated with L-deprenyl, bromocriptine and pergolide in all concentrations used in comparison with that treated with lead acetate alone. This work represented a new trial for protection of astrocytes against toxic effects of lead in vitro.

Abbreviations: CNS: Central nervous system; LDH: Lactate dehydrogenase; GssG: glutathione disulfide; bFGF: basic Fibroblast growth factor; GFAP: Glial fibrillary acidic protein.

Key words: *Dopamine agonists - lead toxicity.*

INTRODUCTION

Despite several decades of research on lead neurotoxicity and its continued prominence as a major environmental and occupational health hazard, the mechanism of its action in the nervous system are still not quite understood. The differential effects of lead exposure in children and adults, as well as inconsistencies between in vivo and in vitro studies, suggest that lead toxicity may have multiple mechanisms in the CNS. Two are: neurodevelopmental toxicity, possibly interference with cell adhesion molecules resulting in miswiring of the CNS during early development and possibly permanent dysfunction; and neuropharmacological toxicity which might involve interactions between lead and both calcium and zinc resulting in interference with neurotransmission at the synapse (Silbergeld, 1992).

Lead is known to induce biochemical alterations in astroglia in the absence of morphological evidence of cytotoxicity and has the potential for

producing molecular and functional damage (Tiffany-Castiglioni et al., 1989). Astroglia carry out many important supportive roles in the brain including potassium spatial buffering of the intercellular environment (Dermietzel et al., 1991), neurotransmitter uptake and intercellular communication (Anders, 1988). Damage to astroglia impair one or more of their supportive functions. For example, exposure to as little as 0.25 µM lead acetate for 7 days significantly reduced the activity of glutamine synthetase in astroglia (Sierra and Tiffany-Castiglioni, 1991). Because astroglia serve a key role in glutamate inactivation via glutamine synthetase, impairment of glutamine synthetase activity may be deterimental to neuronal function.

A line of evidence suggests astrocytes to be an important contributor of an monoamine oxidase- type B (MAO-B) activity in the normal as well as the lesioned CNS (Aquilonius et al., 1992). However, knowledge about the regulation of MAO-B expression in astroglial cells under normal and pathological conditions is at present very limited. Ekblom and Co-workers (1993) reported that the occurrence of MAO-B enzyme protein depends on the degree of cellular differentiation as demonstrated by studies on astrocytes primary cultures which analyzed at two different stages of maturation. Highly differentiated cells exhibited high relative enzyme concentration whereas glioblasts lacked or showed very low contents of MAO-B enzyme.

L-Deprenyl is a selective inhibitor of MAO-B, an enzyme predominantly localized in astrocytes. Biagini et al (1993) investigated the effect of treatment with L-deprenyl (0.25 mg/kg/day) on GFAP immunoreactivity after lesioning the rat striatum with an injection cannula. A significant increase in GFAP immunoreactivity was found in the tissue surrounding the lesion in striata of rats treated with L- deprenyl for 4 days after the lesion. When post- treated for 42 days, however, L- deprenyl no longer increased GFAP immunoreactivity although reactive astrocytes were still present in the lesioned area. These results suggest that L- deprenyl can enhance the activation of astrocytes during a critical time- period following a striatal injury.

Pergolide is a dopamine receptor agonist which acts at both D_1 and D_2 receptors in the nigrostriatal regions of the brain where it effects locomotor activity, exemplified by circling behaviour in lesioned rats. It stimulates adenylate cyclase activity via D1 receptors in the corpus striatum in a manner similar to dopamine. Studies in animals have shown that pergolide can affect corticosterone levels, cardiovasascular function,

prolactin release and intraocular pressure, as well as locomotor activity and stereotypic motor behaviour (Langtry and Clissold, 1990).

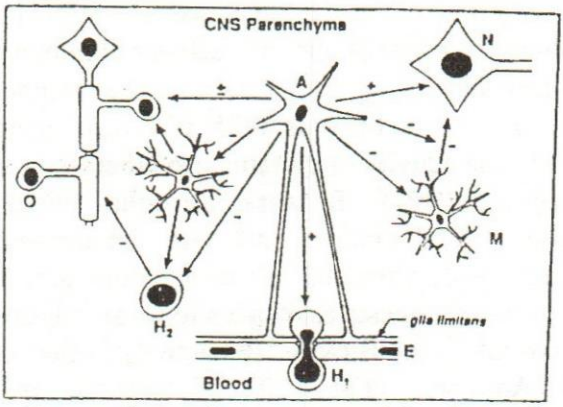

Fig. 1: Interactions of astrocytes with other cells. The production of trophic factors and removal of toxins by astrocytes (A) provides an optimal milieu for neurons (N), oligodendrocytes (O) and other CNS cells. During disease processes, astrocytes may actively recruit hematogenous cells (H_1) into the CNS and modulate the activity of these cells following infilteration of the neural parenchyma (H_2). These astroglial functions could help counteract the potentialy detrimental effects of activated microglia (M). (+) indicates potential enhancing/supporting; (-) indicates potential inhibitory/detrimental effects (Mucke and Eddleston, 1993).

The present study was undertaken to: (1) quantify the morphological alterations (area and number of cells, number and length of processes both primary and secondary) and enzymatic changes (LDH activity in astrocytes that might occur after exposure to low levels of lead (10 μM) for one day. (2) to investigate the protective action of some dopamine agonists (Pergolide, bromocriptine and L-deprenyl) against lead toxicity in these astroglial cultures. Astrocytes from newlyborn C57BL/6 mice were chosen for this study concerning the highly sensitively of them to lead.

MATERIALS and METHODS

1. Materials:

Minimum Essential medium Eagle (MEME) as a basic medium, glucose, corticosterone, putrescin dihydrochloride progesterone, Triiodo thyronine (T3), diaminobenzidine tetrahydrochloride (DAB), pergolide, bromocriptine and L- deprenyl from sigma. Anti- GFAP, fetal calf serum, bovine serum albumin (BSA), L- glutamine, insulin- transferrin -sodium selenite, transferrin, penicillin streptomycin, triton X-100, poly-D-lysine and

LDH diagnostic kits were purchased from Boehringer Mannheim Germany. Sodium bicarbonate, pehydrol and tris buffer were obtained from Merck. Vectastain ABC kits (Vector laboratories). Tissue culture multiwell plates and petri dishes were obtained from Falcon Primaria and Nunc.

2. Methods:

2.1. Cell culture:

Primary astrocyte cultures were prepared from cerebral cortex of newborn C57BL/6 mice (Olson and Holtzman, 1980). The cells were grown in MEME which contain 20% fetal calf serum upto the fourth day of culturing. Then, the medium changed to N_4-medium (serum free medium). This medium was prepared from 50 ml MEME and N_3 (containing BSA 10 µg/m, T_3 10 ng/ml, putrescin 32 µg /ml, corticosterone 2 µg/ml and progesterone 12.5 ng/ml) and insulin (0.01 mg/ml), transferrin (0.1 mg/ml) and sodium selenite (0.004 g/ml). N_4-medium was used another 10 days until treatment was begun by using lead acetate (10 µM) alone or with pergolide, bromocriptine and L-deprenyl (1, 10 and 100 µM) for 24 hours.

2.2. Immunocytochemistry of astrocytes:

Control, lead exposed and lead exposed cultures with pergolide, bromocriptin and L-deprenyl were stained for GFAP as previously described (Olson and Holtzman, 1980), by an adaptation of an immunoperoxidase method (Taylor and Burns, 1974).

2.3. Determination of the morphological changes by video imaging technique:

Pictures of the individual cells were recorded with a Rasterops video cord and a computer (Macintosh SI II) with a Phillips B/W microscopical video camera mounted on NIKON microscope at 200X magnification. Individual pictures were calculated using image 1.41 for cellular area and length. For pixel calculation, calibration were performed by using µm standard length or μm^2 standard areas under the microscope.

2.4. LDH activity assay:

LDH was measured by using a LDH diagnostic kit. Aliqouts of the supernatant from the control and treated cultures were tested immediately for the presence of LDH as a measure of cytoplasmic leakage (Bergmeyer and Bernt, 1974). The analysis was carried out by using an Eppendorf digital photometer 61145.

2.5. Statistical analysis:

For each individual experiment, values of the different groups were calculated for summary statistics (mean valuse ± standard error) using

student›s t test. A probability value 0.05 was considered to be signifcantly different from control and lead acetate treated groups and cultures treated also with L-deprenyl, bromocriptine and pergolide in the morphological changes and was 0.001 in LDH activity.

RESULTS

1- Morphometry:

The morphological changes of astrocytes from the cerebral cortex were measured. A significant difference was found between lead acetate treated groups and control groups and between lead acetate treated groups and groups treated with dopamine agonists (in all concentrations which were used) specially in the area and number of astroglia (Table 1). In case of number of primary processes, a significant difference was observed only in 10 μM pergolid, 100 μM bromocriptine and 1 μM deprenyl, while no significant difference was observed in their length either between control and lead acetate treated groups or between lead acetate treated groups and any groups treated with dopamine agonists (Table 1). A significant difference was found between control and lead acetate groups in case of number of secondary processes and also in their length, while between lead acetate treated groups and 10,100 μM bromocriptine treated groups for their number and with 10, 100 μM for bromocriptine and with all concentrations of deprenyl for their length (table 1).

2- LDH activity:

LDH activity in cultures treated with 1, 10, 100 μM of L-deprenyl, bromocriptine and pergolide was significantly reduced ($P<0.001$) in comparison with cultures treated with 10 μM lead acetate, although lead acetate treated cultures was signifcantly increased than that untreated one (Table 2).

DISCUSSION

The efficacy of dopamine agonists (L-deprenyl, bromocriptine and pergolide) in reducing the toxic effects of lead on astrocytes prompted us to examine this efficacy. Our study shows that dopamine agonists could significantly lowering the effect of lead on in vitro astrocytes (Tables 1&2).

bEGF increases neuronal survival and growth in cell cultures and stimulates functional recovery from brain lesion. In addition, bFGF is able to induce glial cell proliferation and differentiation. Recently, L-deprenyl has been shown to potentiate astrocyte reaction to a mechanical lesion and to

possess a trophic-like activity in several experimental models. Biagini and Co-workers (1994) investigated the effect of L-deprenyl (0.25 mg/kg/day) on bFGF immunoreactivity after the insertion of an injection cannula in rat neostriatum. They have been found that subchronic L-deprenyl treatment potentiates both the lesion-induced increase of GFAP and bFGF. These findings suggest that a possible mechanism for L-deprenyl induced neuroprotection may be the activation of astrocytes associated with increased secretion of trophic factors that promote neuronal survival and growth. This astrocyte-kinetic action of L-deprenyl could represent a new therapeutical approach to increase trophic support of lesioned neurons.

Several neurochemical characteristics of the substaintia nigra may enhance free radical formation and contribute to oxidative stress vulnerability. Dopamine can be oxidatively metabolized by the enzyme MAO. The polymerization of auto-oxidative products of dopamine leads to the formation of neuromelanin and the characteristic pigmentation of the substania nigra. Both outo-oxidation of dopamine and oxidative deamination by MAO result in the formation of hydrogen peroxide (H_2O_2). Under normal circumstances H_2O_2 is rather inert and never accumulates in the brain or other organs. H_2O_2 is normally cleared from the brain by the glutathione system. Glutathione peroxidase catalyzes the reaction of H_2O_2 with glutathione (GSH) to form GssG:

H_2O_2 + 2GSH-(in presence of Glutathione peroxidase) $\rightarrow 2H_2O$ + GssG. In the persence of iron, H_2O_2 can be reduced to form the toxic hydroxyl free radical (Fenton reaction): $H_2O_2 + Fe^{2+} \rightarrow 0H + 0H^- + Fe^{3+}$. MAO activity in the brain with ageing (Fowler et al, 1980) and this may lead to an increase in the formation of H_2O_2 which could exceed the capacity of the glutathione system. Similarly, a reduction in glutathione or glutathione peroxidase could prevent the clearance of H_2O_2 generated from normal dopamine metabolism (Sofic et al., 1992).

Dopamine can play a neurotoxic role in ischaemic brain damage. pergolide is not only a potent dopamine agonist at postsynaptic dopamine receptorts but also a potent agonist at presynaptic dopamine autoreceptors (Fuller et al., 1982). Pergolide given daily I.P. for three weeks at 0.04 mg/kg and 0.4 mg/kg, significantly induced soluble (Cu-Zn) superoxide dismutaste in the rat striatum, while having no effect on the mitochondrial (Mn) form of

the enzyme. Such induction, which can also be affect by L- deprenyl, may help to protect against nigrostriatal degeneration (Clow et al. 1992).

Pergolide initially increases serum corticosterone levels in rats, possibly via central dopamine receptors. Pergolide 7.5 µg/Kg reduces blood pressure in normotensive dogs and larger reductions of blood pressure occur in hypertensive animals. Similarly, heart rate, blood pressure, plasma dopamine, norepinephrine and lactic acid levels are reduced at rest and/or during exercise when pergolide 0.05 mg/ day is administered to health volunteers. Pergolide appears devoid of effects on glucose metabolism in either animals or patients with parkinson›s disease. Furthermore, it reduces intraocular pressure in healthy volunteers and causes significant reductions in prolactin at rest and during exercise (Langtry and Clissold,1990). Pergolide was also used to treat cushing›s syndrome or hyperadrenocorticism (which result from long term overproduction of cortisol by the adrenal cortex in horse (Munoz et al, 1996).

CONCLUSION

The present results demonstrate a new possible protective action of various dopamine agonists (L-deprenyl, pergolide and bromocriptin) on in vitro intoxicated astrocytes that they potentiate lesion-induced astoglial activation. In the view of trophic role of glial cells on neurons, this effect may be important to the neuroprotective action of these dopamine agonists in some models of brain toxicity

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Table 1. Effect of lead acetate exposure on astroglia in presence of pergolide, bromocriptine and L- deprenyl (meam ± S.E.M.)

Treatment	Area (um^2)	Number of cells/cm^2	Number of primary proc./cell	Length of primary proc.,µm	Number of sec. proc./cell	Length of sec. proc., µm
Control	4921 ± 226	39.9 ± 1.7	9.0 ± 0.7	45 ± 3	8.7 ± 0.8	27 ± 2
10 µM lead acetate	1573 ± 109[a]	13.9 ± 0.7[a]	5.4 ± 0.7[a]	40 ± 4	3.0 ± 0.9[a]	16 ± 3[a]
A + 1 µM pergolide	2652 ± 120[a,b]	23.6 ± 1.0[a,b]	6.5 ± 0.6[a]	39 ± 3	4.7 ± 0.8[a]	20 ± 3[a]
A + 10 µM pergolide	3489 ± 162[a,b]	24.3 ± 1.1[a,b]	7.6± 0.7[b]	42 ± 2	4.2 ± 0.6[a]	19 ± 2[a]
A + 100 µM pergolide	3436 ± 201[a,b]	25.2 ± 0.9[a,b]	6.6 ± 0.3[a]	42 ± 3	5.2 ± 0.7[a]	22 ± 3
A + 1 µM bromocriptine	3161 ± 208[a,b]	24.7 ± 1.3[a,b]	5.9 ± 0.5[a]	41 ± 3	4.4 ± 0.5[a]	21 ± 2[a]
A + 10 µM bromocriptine	2913 ± 109[a,b]	24.5 ± 1.1[a,b]	6.8 ± 0.4[a]	43 ± 2	5.0 ± 0.5[a,b]	26 ± 2[b]
A + 100 µM bromocriptine	2734 ± 162[a,b]	25.0 ± 1.1[a,b]	8.4± 0.9[b]	44 ± 3	5.9 ± 0.8[a,b]	28 ± 2[b]
A + 1 µM L-deprenyl	2875 ± 148[a,b]	24.5 ± 1.1[a,b]	8.0± 0.6[b]	45 ± 3	4.9 ± 0.8[a]	27 ± 2[b]
A+ 10 µM L-deprenyl	3055 ± 239[a,b]	25.2 ± 1.1[a,b]	7.4 ± 0.7	45 ± 3	4.9 ± 0.5[a]	24 ± 1[a,b]
A+ 100 µM L-deprenyl	3485 ± 255[a,b]	23.9 ± 1.0[a,b]	6.7 ± 0.4[a]	48 ± 3	5.1 ± 0.7[a]	23 ± 1[a,b]

[a] Significantly different from control (non treated cultures), $p< 0.05$.
[b] Significantly different from lead acetate treated groups, $p< 0.05$.
A = 10 µM lead acetate.

Table 2. LDH release (nkat/ml) due to exposure to lead acetate alone or in presence of various dopamine agonists (mean ± S.E.M.)

Treatment	LDH release	LDH release (% of lead acetate expesed group)
Control	93 ± 12	
10 µM PbAc	672 ± 35[a]	100 ± 5.2
A + 1 µM pergolide	248 ± 17[a,b]	36.9 ± 2.5
A + 10 µM pergolide	252 ± 10[a,b]	37.5 ± 1.4
A + 100 µM pergolide	284 ± 30[a,b]	42.2 ± 4.4
A + 1 µM bromocriptine	287 ± 25[a,b]	42.7 ± 3.7
A + 10 µM bromocriptine	264 ± 6[a,b]	39.2 ± 0.8
A + 100 µM bromocriptine	237 ± 14[a,b]	35.2 ± 2.0
A + 1 µM L-deprenyl	289 ± 17[a,b]	43.0 ± 2.5
A + 10 µM L-deprenyl	301 ± 23[a,b]	44.7 ± 304
A + 100 µM L-deprenyl	246 ± 16[a,b]	36.6 ± 2.3

[a] Significantly different from control supernatant, $p < 0.001$.
[b] Significantly different from lead acetate treated groups, $p < 0.001$.
A = 10 µM lead acetate.