Korean J. Zool., 32:34-39, 1989

CNS Durg-induced Redistribution of Lactate Dehydrogenase Isozymes in Mice

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Ouabain, strychnine sulfate, caffeine sodium benzoate and chlorpromazine hydrochloride were introduced intraperitoneally into male mice for 7, 14 and 21 days to induce the changes in the relative percentages of lactate dehydrogenase isozymes. The five isozymes in brain, heart and kidney tissues were electrophoresed on cellulose acetate strip and subjected to densitometry.

Ouabain caused a drastic increase of B_4 isozymes only in brain tissues. The two stimulants altered the relative percentages of A_4 and B_4 isozymes conspicuously in brain tissues, whereas virtually no redistributions of five isozymes were occurred by the depressant except B_4 isozymes in brain and heart tissues.

On the basis of these observations, it might be suggested that the changes in intracellular concentration of sodium and calcium ions are not the cause of the isozyme redistributions and that organization of plasma membrane could be one of the factors involved in the tissue specificity of lactate dehydrogenase isozymes in vertebrates.

KEY WORDS: Lactate dehydrogenase, Stimulant, Depressant, Plasma membrane

The evolutionary tendency of lactate dehydrogenase (EC 1.1.1.27; LDH) in vertebrate species towards forming electrophoretically five distinct isozymes could be derived from the facts that specific heterotetrameric isozyme(s) can not be found in poikilotherms (Markert and Faulhaber, 1965), even though there are lots of A and B subunits in their cytoplasmic environments.

Furthermore, the purified A_4 and B_4 isozymes from five-isozyme typed species could make three heterotetramers after freezing-thawing in NaCl (Markert, 1963), while those isozymes from species having only A_4 and B_4 isozymes would not do (Yum *et al.*, 1981).

This might mean that the amino acid sequence of binding sites on the two subunits has been drastically changed during the course of vertebrate evolution due to certain physiological needs for five isozymes.

In an attempt to elucidate a precise mechanism by which quaternary structure formation is brought about, artificial *in vivo* alterations in the relative percentage of the LDH isozymes have been made by change in oxygen tension (Mager *et al.*, 1968), poisoning by heavy metals (Secchi *et al.*, 1970), hormonal treatment (Suleiman and Vestling, 1979), exposure to chemical carcinogens (Spence, 1979), cold acclimatization (De Costa *et al.*, 1981) and blocking the sodium channels (Kim and Yum, 1985).

One of the most reliable ways to study the formation mechanism of quaternary structure is to perturb the intracellular environment through getting cells exposed to chemicals which might change energy metabolism or membrane struc-

This work was supported by the Research Grant from the Korea Science and Engineering Foundation.

ture.

In the present work with mouse LDH isozymes in which ouabain, two stimulants strychnine and caffeine, and a depressant chlorpromazine (CPZ) were used for the purpose of interrupting the cellular environment, we have observed several characteristics of alteration in the isozyme distribution which might be one of the clues to the mechanism of the quaternary structure formation of the protein.

Materials and Methods

Albino male mice weighing approximately 40 g were kept in five groups of 12 each. The first was used as control. The second to fifth were subjected to multiple intraperitoneal injections of ouabain (10 mg/Kg/d), strychnine sulfate (0.1 mg/Kg/d), caffeine sodium benzoate (25mg/Kg/d) and chlorpromazine hydrochloride (1mg/Kg/d), respectively.

Three individuals from each group were sacrificed after 7, 14 and 21 days of the *in vivo* chemical exposure. After decapitation, brain, heart and kidney tissues were used in the electrophoresis.

Each tissue was ground in an ice-bathed glass homogenizer in cold demineralized water followed by centrifugation in a PR-2 International refrigerated centrifuge at 6,000 $\times$g for 1 hr. The resulting crude extracts were used within 4 days. Electrophoresis on cellulose acetate strip (Millipore, ESWPO25FT) was carried out by the methods of Yum *et al.* (1981).

Zymograms after the electrophoresis were subjected to densitometry with an automatic computing densitometer (Gelman, ACD-18). The results were expressed as mean of at least triplicate experiments.

The four pharmacological drugs and NAD were obtained from Sigma and the buffer components barbituric acid and sodium barbiturate from Merck. All chemicals used were of highest reagent grade available.

Results and Discussion

Ouabain

By using the cultured human cells, Lamb and McCall (1972) described that prolonged ouabain treatment caused increase in $[Na]_i$ and decrease in $[K]_i$. The elevation in $[Na]_i$ could produce concomitant rise in $[Ca]_i$. Indeed, ouabain inhibits parathyroid hormone release out of bovine parathyroid cells (Rothstein *et al.*, 1982).

Table 1 summarizes the conspicuous increase of B_4 isozyme in mouse brain tissues, after exposure to ouabain, to such an extent that nearly all experimental brain tissues have twice as much B_4 isozyme as control brain. This result might be comparable with the sharp increase of brain B_4 isozyme in mouse exposed to tetrodotoxin which blocks the membrane sodium channels (Kim and Yum, 1985).

It is, thus, reasonable to speculate that neither the depletion of intracellular sodium ion nor the increase in intracellular sodium ion is direct cause of the change in relative percentage of five LDH isozymes.

Contrary to brain tissues, no drastic changes in

Table 1. Effects of *in vivo* multiple treatment of ouabain (10mg/Kg/d) on the relative percentage of five LDH isozymes in mouse tissues.

Days	Relative percentage					A/B
	A_4	A_3B	A_2B_2	AB_3	B_4	
Brain						
Control	14.0	27.4	31.4	17.5	9.7	1.20
7	11.9	23.5	20.4	19.7	24.5	0.81
14	13.0	22.8	23.8	18.4	22.0	0.87
21	14.9	20.3	18.2	19.8	26.8	0.79
Heart						
Control	20.1	19.5	27.0	20.6	12.8	1.14
7	22.4	18.7	23.1	20.3	15.5	1.13
14	21.3	20.4	20.2	20.5	17.6	1.08
21	17.3	16.3	23.1	23.4	19.9	0.88
Kidney						
Control	11.0	24.0	25.2	23.5	16.3	0.90
7	25.0	21.3	21.4	17.4	14.9	1.27
14	17.4	22.1	21.2	19.6	19.8	0.98
21	14.9	23.9	21.6	18.8	20.8	0.94

A/B ratio can be met in heart and kidney tissues. The differences in sensitivity of these three tissues to ouabain might be explained by membrane lipid biochemistry. Several lines of evidence demonstrate that the relative potency of the cardiac glycosides for (Na + K)-ATPase inhibition is highly dependent on the source of the enzyme (Akera, 1971; Marks and Seeds, 1978). Rat heart (Na + K)-ATPase was markedly less sensitive to ouabain or digoxin than was rat brain or guinea pig heart enzyme (Akera *et al.*, 1985). Those characteristic affinity of the enzymes for the glycoside has been explained in terms of lipid biochemistry that the constituent membrane fatty acids and cholesterol play an important role as possible modulators of the inhibitory action of cardiac glycosides (Abeywardena and Charnock, 1983; Yeagle, 1983).

Strychnine

Table 2 demonstrates the sharp increase of B_4 isozyme in mouse brain tissues after exposure to strychnine. The relative percentage of A_4 isozyme in brain and heart tissues diminishes, while that in kidney tissues increases. No significant changes were found in three heterotetramers.

The binding of glycine to the postsynaptic glycine receptor produces an inhibitory effect on neuronal firing that is mediated by an increase in chloride conductance and this effect of glycine is antagonized by the alkaloid strychnine (Curtis *et al.*, 1971).

Strychnine binding site is known to associate with only one polypeptide of glycine receptor which contains three major polypeptides. Peptide mapping of this strychnine binding subunit of purified pig and rat glycine receptor showed that those binding sites are highly conserved between these species (Graham *et al.*, 1985).

It is, thus, highly acceptable that the isozyme redistribution might be resulted from the membrane reorganization caused by strychnine binding to the glycine receptor which in turn affects the conformation of membrane proteins including those of sodium channels.

Caffeine

Caffeine (1,3,7-trimethylxanthine) is known to have pleotropic effects at the cellular level. Exposure of mammalian skeletal muscle to the alkaloid, which readily penetrates the sarcolemma, produces a phasic contracture which has been shown to result from a release of calcium ions from the sarcoplasmic reticulum (Weber and Herz, 1968). This caffeine contracture is reported to be produced in cardiac muscles of various mammalian species (Chapman and Leoty, 1976). Caffeine has been reported, furthermore, to increase the sensitivity of the contractile proteins in cardiac muscle to calcium ion (Eisner and Valdeolmillos, 1985).

No significant changes in the relative percentages of five LDH isozymes can be met in heart tissue (Table 3), suggesting that the intracellular concentration of calcium ion does not play a role in the process of quaternary structure formation.

Caffeine in the brain tissue made a significant increase of the B_4 isozyme, while it did a decrease of A_4 isozyme, being similar changes in brain tissue exposed to a stimulant strychnine.

Despite the vast amount of data on different effects of caffeine, its molecular mechanism of action is not known. The involvement of caffeine with the inhibition of enzyme activity is well documented. Caffeine is known to inhibit 5′-nucleotidase activity in brain (Tsuzuki and Newburgh, 1975), kidney (Fredholm *et al.*, 1978) and heart (Heyliger *et al.*, 1981). Thus, the pro-

Table 2. Effects of *in vivo* multiple treatment of strychnine (0.1mg/Kg/d) on the relative percentage of five LDH isozymes in mouse tissues.

Days	Relative percentage					A/B
	A_4	A_3B	A_2B_2	AB_3	B_4	
Brain						
Control	14.0	27.4	31.4	17.5	9.7	1.20
7	11.1	25.5	28.0	18.6	16.8	0.96
14	11.5	27.5	27.6	18.8	14.6	1.03
21	8.7	24.4	30.0	19.1	17.8	0.88
Heart						
7	20.1	19.5	27.0	20.6	12.8	1.14
14	10.8	18.3	27.5	26.9	16.5	0.82
21	13.9	21.4	32.6	23.8	8.3	1.09
Kidney						
Control	11.0	24.0	25.2	23.5	16.3	0.90
7	16.6	20.9	25.5	23.3	13.7	1.03
14	13.6	19.5	24.5	23.4	19.0	0.86
21	16.9	21.5	24.2	22.7	14.7	1.03

Table 3. Effects of *in vivo* multiple treatment of caffeine(25mg/Kg/d) on the relative percentage of five LDH isozymes in mouse tissues.

Days	Relative percentage					A/B
	A_4	A_3B	A_2B_2	AB_3	B_4	
Brain						
Control	14.0	27.4	31.4	17.5	9.7	1.20
7	13.0	21.3	27.8	20.6	17.3	0.92
14	7.7	25.3	25.1	22.3	19.6	0.81
21	8.8	22.6	27.8	20.0	20.8	0.81
Heart						
Control	20.1	19.5	27.0	20.6	12.8	1.14
7	23.4	18.9	23.8	21.1	12.8	1.21
14	22.9	19.4	24.9	20.5	12.3	1.22
21	16.6	19.7	23.8	21.3	18.6	0.95
Kidney						
Control	11.0	24.0	25.2	23.5	16.3	0.90
7	18.5	22.2	25.3	21.7	12.3	1.14
14	15.7	21.7	25.0	24.1	13.5	1.02
21	17.3	22.8	24.5	21.4	14.0	1.08

Table 4. Effects of *in vivo* multiple treatment of chlorpromazine (1mg/Kg/d) on the relative percentage of five LDH isozymes in mouse tissues.

Days	Relative percentage					A/B
	A_4	A_3B	A_2B_2	AB_3	B_4	
Brain						
Control	14.0	27.4	31.4	17.5	9.7	1.20
7	10.8	22.7	27.6	22.3	16.6	0.89
14	14.7	26.9	28.5	18.	11.1	1.17
21	11.5	27.0	29.2	20.3	12.0	1.06
Heart						
Control	20.1	19.5	27.0	20.6	12.8	1.14
7	17.4	29.6	27.6	19.3	6.1	1.40
14	12.2	23.5	28.7	23.8	11.8	1.00
21	15.0	26.4	26.7	20.0	11.9	1.13
Kidney						
Control	11.0	24.0	25.2	23.5	16.3	0.90
7	13.9	22.4	24.1	22.7	16.9	0.94
14	15.5	20.4	24.6	24.9	14.6	0.97
21	11.4	21.8	26.6	23.5	16.7	0.88

duction rate of adenosine seems to have nothing to do with the physiological need of five LDH isozymes.

Chlorpromazine

After 7 days of CPZ exposure, brain tissue shows comparatively low A/B ratio, whereas heart tissues comparatively high A/B ratio (Table 4), suggesting that the B_4 isozymes in two tissues are different from each other at a viewpoint of physiological behavior.

The pharmacological effects of CPZ is due primarily to the blocking of dopamine receptors (Tamir *et al.*, 1981). A number of mechanisms have been demonstrated with regard to the effects of CPZ on biological membranes. At low concentrations, CPZ has been shown to strongly stabilize the erythrocyte membrane against hypotonic hemolysis (Seeman and Kwant, 1969). In mitochondria, CPZ has been shown to protect the membrane at pH 7.9 against the action of exogenously added phospholipase A (Seppala *et al.*, 1971). On the other hand, CPZ and each of its metabolites alter liver plasma membrane lipid fluidity (Keeffe *et al.*, 1980).

Membrane and B_4 isozyme

On the basis of our observations that, depending on the kind of tissues, the relative percentages of five tetramers could be altered to different extent by exposing those tissues to chemicals which affect the membrane structure, it seems likely that intracellular environments have to be continuously controlled to maintain their low entropy.

Furthermore, it should be noted that cells of central nervous system reveal physiological need for B_4 isozyme when they are exposed to unaccustomed environment. Everse and Kaplan (1975) proposed the hypothesis that the A type LDH is especially geared to serve as a pyruvate reductase in anaerobically metabolizing tissues, whereas B type enzyme is better suited to serve as a lactate dehydrogenase in aerobically metabolizing itssues. Thus, it might be acceptable that NADH is needed in the experimental brain tissue to cope with the increase of intracellular entropy induced by changes of membrane structure and function.

It is fairly well established that temperature itself causes a change in the properties of membrane lipids which tends to offset the perturbing effects of changed cellular temperature and thereby to preserve the physical state of those lipid compart-

ments in some optimal and advantageous condition (Cossins and Prosser, 1982). Hill and Bangham (1975) suggested that a lipid-soluble drug might enter the membrane, disrupting the normal membrane organization and thus making the structure more fluid and that the cell responds by incorporating straighter phospholipids which are less fluid into its membrane. Although it is still in doubt as to whether the homeoviscous drug-adaptation has relevance to our intracellular isozyme redistribution, it is certain that an emphasis has to be on the potentially more specific effects of lipids on, for example, membrane protein conformation as pointed out by Littleton (1983).

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(Accepted December 25, 1988)

CNS drug에 의한 mouse 젖산수소이탈효소 동위효소의 재분포

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젖산수소이탈효소 동위효소의 상대적 값(%)을 변화시키기 위하여 ouabain, strychnine sulfate, caffeine sodium benzoate 및 chlorpromazine hydrochloride를 웅성 mouse에 7일, 14일 및 21일간 복강주사하였다. 뇌, 심장 및 신장조직의 이 효소를 셀룰로우즈 strip상에서 전기영동한 후 densitometry에 의하여 동위효소들의 상대적 값(%)을 구하였다.

Ouabain은 뇌조직에서만 B_4동위효소를 급격히 증가시켰다. 두가지 stimulant들은 뇌조직에서 A_4 및 B_4 동위효소의 상대적 값(%)을 뚜렷하게 변화시킨 반면 depressant는 뇌조직과 심장조직의 B_4 동위효소를 제외하고는 동위효소의 재분포를 유도시키지 않았다.

이러한 실험적 결과들로부터 Na^+이온이나 Ca^{++}이온들의 세포질내 농도변화는 젖산수소이탈효소 동위효소의 재분포의 원인이 되지 않으며 원형질막의 체제가 이 효소의 조직특이성을 나타나게 하는 한가지 요인이 될 수 있다고 사료된다.