МЕДИЦИНСКИЕ НАУКИ

РАССТРОЙСТВА СОКРАТИМОСТИ В ГЛАДКО - МЫШЕЧНОЙ ТКАНИ ЖЕЛУДКА ИНДУЦИРОВАННЫЕ СВИНЦОМ

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LEAD-INDUCED CONTRACTILITY DISORDERS IN GASTRIC SMOOTH MUSCLE TISSUE

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АННОТАЦИЯ

В исследование рассматриваются специфические эффекты аномальной концентрации свинца в кровотоке (отравление свинцом) в желудочно-кишечном тракте (ЖКТ). Исследованы изменения сократительной способности и тонуса гладкой мышечной ткани ЖКТ для концентраций свинца выше 700 мкг/л. Предлагается механизм взаимодействия, который связывает ионы свинца с синтезом простагландинов.

ABSTRACT

This study examines specific effects of abnormal lead concentration in the bloodstream (lead poisoning) on the gastrointestinal tract (GIT). Investigated are changes in the contractility and the tonus of GIT smooth muscle tissue for lead concentrations above 700 μg/l. An interaction mechanism is suggested that links lead ions to the prostaglandin synthesis.

Ключевыеслова: Pb^{2+}; желудочно-кишечный тракт; гладко-мышечная ткань; in vitro.

Keywords: Pb^{2+}; gastrointestinal tract; smooth muscle tissue; in vitro.

Introduction

Toxicity of lead after ingestion or inhalation has been well established. Accumulation above certain levels has been linked to disorders of the cardiovascular system [9,17,22], the musculoskeletal system [26], the excretory system [25], the reproductive system, and the nervous system [2,23] .

Previous studies of the effects of lead on the gastrointestinal tract (GIT) have addressed issues related to acute exposure such as nausea, vomiting, and spastic abdominal pain [15], that are traditionally associated with the contractile function of intestinal smooth muscle tissue.

Effects on the tonus of GIT smooth muscles (SM) caused by chronic high concentration of lead in the bloodstream are not well understood, and proposed mechanisms are often contradictory.

Studies on the probable interaction paths of Pb^{2+}on cellular level include inhibition or mimicking of the effects of Ca^{2+}(effects on all Ca^{2+}-related cellular processes) [24,14]; interaction with proteins such as sulfhydryl, amine, phosphate and carboxyl groups

[12,19]; effects on the synthesis and secretion of neurotransmitters [20]; and modulation of NO production [13]. Most studies have been based on SM tissue from blood vessels [21,18] or bronchial tubes [16].

Materials And Methods

Patients and laboratory animals

The GIT radiographic study was conducted with 48 patients, who had lead concentrations in the blood serum above 700 μg/l. The control group consisted of 169 patients with upper dyspeptic syndrome symptoms, scheduled for contrast radiography of the GIT. Patients in this group had not been exposed to Pb^{2+}, and had normal lead concentrations in the bloodstream.

All participants in the study were detailed on the aim and methods of the study, and have signed a written participation agreement.

The in vitro experiments were conducted with SM tissue from uniformly treated Wistar adult male rats weighting 230-250 g. Rats were grown at temperature 20-22^{o}C, feeding regimen, free access to water, and 12h of daily light exposure. Feeding was discontinued 12-13h before the experiment. Decapitation was done upon admission of ester anaesthesia. Circular SM preparations (length 14-15 mm, width 1.4-1.5 mm) were cut out from the corpus muscle layer. The specific number of SM preparations for each study is listed in the results section. Experimental animals were treated according to Directive 86/609/EEC .

Contrast Radiography of the GIT

The radiographic study (x-ray and fluoroscopy) of the GIT was conducted with Process 800 ST (CGR) and Siemens Sirescop 1500ST units, and Barium contrast agent ($BaSO_4$ suspension in water 1:8, mixed at temperature of 37°C). Examinations were carried out in the morning, on empty stomach, and after blood pressure checkup. Patients were first administered 2-3 sips of the total contrast agent, in upright posture and with application of moderate compression, followed by the ingestion of entire contrast mixture. The contrast agent allowed for visualisation of the size, shape, and position of the stomach in the abdominal cavity, as well as the intestinal tonus and the peristaltic activity. Changes in the intestines were recorded within 24 and 72 hours after the beginning of the observation. The following equipment parameters were used: Voltage: 86 KV, Current:100 mAs, Duration: 0.4 s. The frequency of appearance of radiographically significant changes, associated with particular illnesses (such as gastroduodentis, colitis, or inflammation of the appendix), was measured

Matching of the, size, tonus, and peristaltic activity of the organs of the GIT of patients with high lead concentrations in the bloodstream and the control group were used for determination of specific radiographically detectable Pb-induced effects.

Mechanical activity in smooth muscle preparations

The mechanical activity of smooth muscle tissue was measured via piezoresistive sensors. SM preparations were fixed on one side to a glass holder, and on the other end (via surgical tread) to a piezoresistve strain guage model Swema (Stockholm, Sweden).

During the testing SM tissue preparations were submerged into Krebs solution (pH=7.4, t^{o}=37^{o}C) bath (20 ml) with the following contents (mmol/l): NaCl-120; KCl-5.9; $CaCl_2$ - 2.5; $MgCl_2$ - 1.2; NaH_2PO_4-1.2; $NaHCO_3$-15.4 and glucose-11.5. Acidity of the solution measured with Microcomputer pH-meter 6201 (Jenco Electronics, UK).

The Krebs solution was continuously aerated with a mixture of O_2/CO_2 in fractional volume ratio of 19:1. Mechanical strain calibration of the SM preparations was achieved via tensioning of the system with standard 10 mN force. Within 60 min after tensioning, the tonus and the spontaneous contractile activity of SM preparations stabilized. In this period the Krebs solution was exchanged 2-3 times. Thus the established tonus after adaptation period of 60 min was taken as the initial, or the 0^{th} level against which subsequent mechanical changes were measured. Parameters of the spontaneous contractile activity, such as frequency and amplitude (force) of the contractions, taken before the application of test substances, were set as referential. The character and the magnitude of the Pb^{2+}-induced changes in the spontaneous SM mechanical activity, and the tonus were measured against the corresponding initial values.

Test substances were applied in precise volume concentrations, necessary to achieve target concentrations in the tissue bath. Volumes of added test substances did not exceed 1/100 part of the total solution volume. The vitality of the SM tissue was probed with $1x10^{-6}$mol acetylcholine, twice in the beginning and in the end of each test cycle.

Electrical signals from the strain gauges were amplified using model K. Tesar- D 486 (Germany), or Microtechna (Prague, CzechRepublic) commercial amplifiers. Mechanical activity was recorded with analog recorder model Linseis (Selb, Germany).

Drugs, solutions, and chemicals

During the study the following drugs and chemicals were used: Acetylcholine (Dispersa Baeschlin, Germany); Indomethacin, Tetrodotoxin (TTX), Raclopride (R-121), Methysergide and Hexametonium, Lead(II) acetate, Acetylsalicylic acid, Phentolamine hydrochloride (Sigma Chemical Company, St. Louis, MO, USA); Atropine (Sopharma, Bulgaria); $BaSO_4$ (DC-BAR-Milve, Bulgaria). The Krebs solution had the following content (mM): NaCl 120; KCl 5.9; $CaCl_2$ 2.5; $MgCl_2$ 1.2; NaH_2PO_4 1.2; $NaHCO_3$ 15.4, and glucose 11.5. All substances of the Krebs solution were made by Merck (Darmstadt, Germany).

Statistical analysis

Test data was analyzed using STATISTICA software. Results are presented as average values ± standard deviation. Confidence was based Students’ t-test with level of 95% ($p<0.05$).

Results

1. Radiographic study

The imaging data shows distinguishable differences in the condition of the GIT of the two test groups. Patients with abnormal lead concentrations show higher incidents of colitis and appendix inflammation of the (Fig 1a and 1b), conditions related to the GIT motility. Quantitative data regarding the frequency of occurrence of the aforementioned conditions in patients with high lead concentrations and the control group is shown in table 1. Specific features more frequently observed in the radiographic images of with high lead exposure as compared to the control group, are changes in the tonus of the stomach, visible as stenosis (constriction) of the antral and upper parts of the duodenal arch (Fig 2a), as well as the appearance of deep, segmenting peristaltic. (Fig 2b).

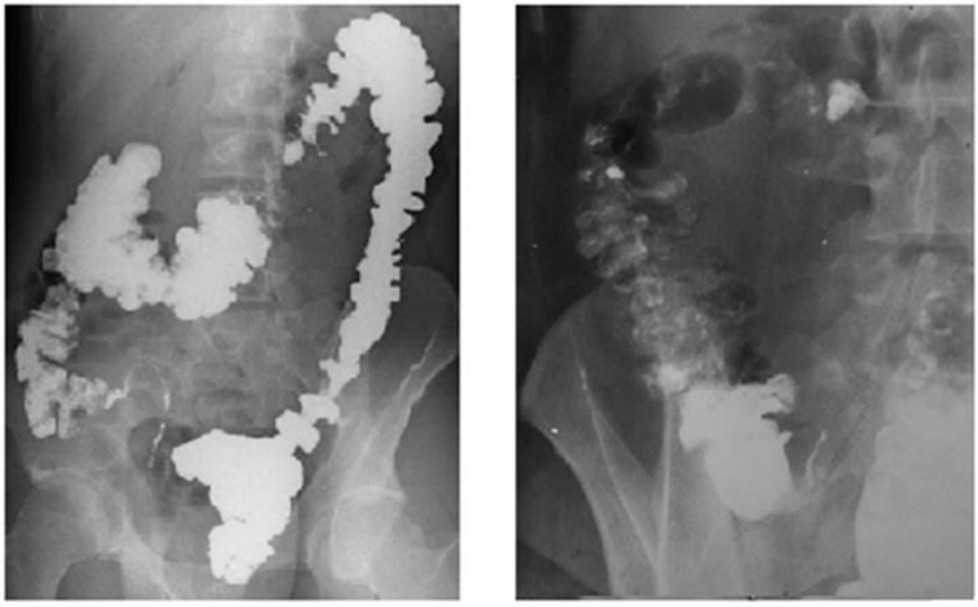

Figure 1. X-ray images of the GIT in patients with high lead exposure:

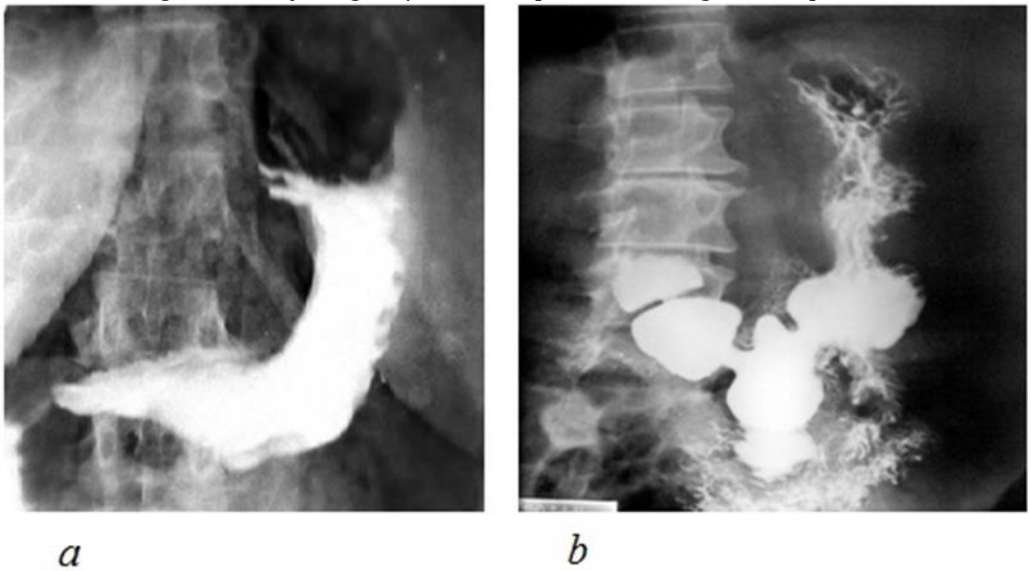

Figure 2. X-ray images of stomach of patient with high lead concentrations, in upright position:
a) Constriction of the antrum and the upper part of the duodenal arch;
b) Deep, segmenting stomach peristaltic.

Table 1. Comparison of the radiographic abnormalities in the GIT for patients with work-related lead aerosol exposure and the control group, not employed in the lead industry.

Radiographic Abnormalities	Control group		Patients with work-related lead exposure		t	p
	n	%	n	%		
Stomach	40	**23.7**	32	**74.4**	6.8	<0.001
Colitis	41	24.2	22	51.1	3.2	<0.01
Appendicitis	7	**4.1**	21	**48.8**	5.7	**<0.001**

2. In vitro study of smooth muscle tissue

Pb^{2+}caused contraction in the stomach SM tissue preparations of rats (Fig 3). The effect is reversible and allows for recovery of the initial contractile parameters of the test samples, after replacement of the Krebs solution.

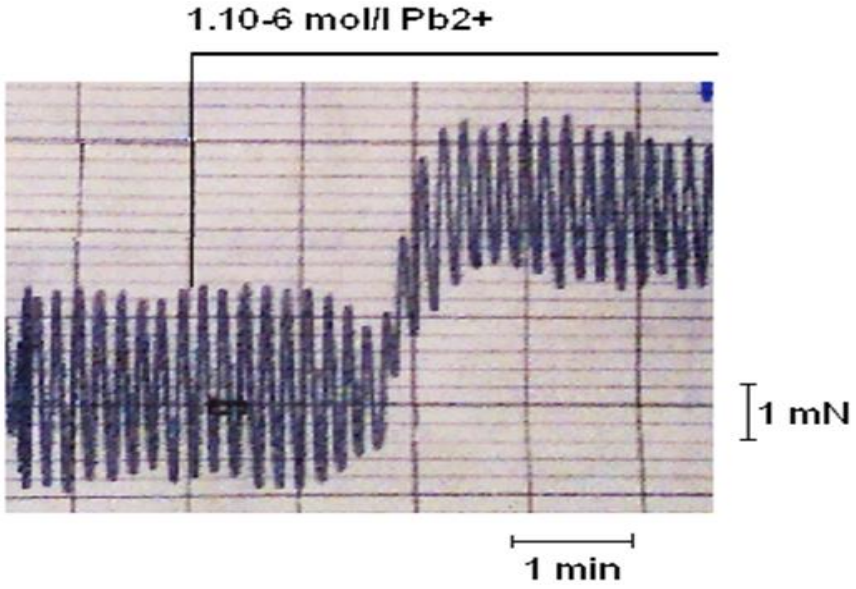

Fig.3.Contractile activity in stomach SM tissue of rat after application of 1.10^{-6} mol/l Pb^{2+}.

The contraction/concentration curve (Fig 4) traces the dependence of muscle tonus on gradually increased Pb^{2+}concentration ($1x10^{-7}$ – $1x10^{-5}$ mol/l). Pb^{2+} concentrations above $1x10^{-4}$mol/l caused non-uniform SM responses (from weak contraction to relaxation, and dual-phase contractions). Application of the latter concentrations for 15-20 min durations causes reduction, and often complete suppression of the spontaneous contractions. Exchange of the Krebs solution in the tissue bath did not lead to recovery of the initial contractile activity of the Pb^{2+}-treated preparations.

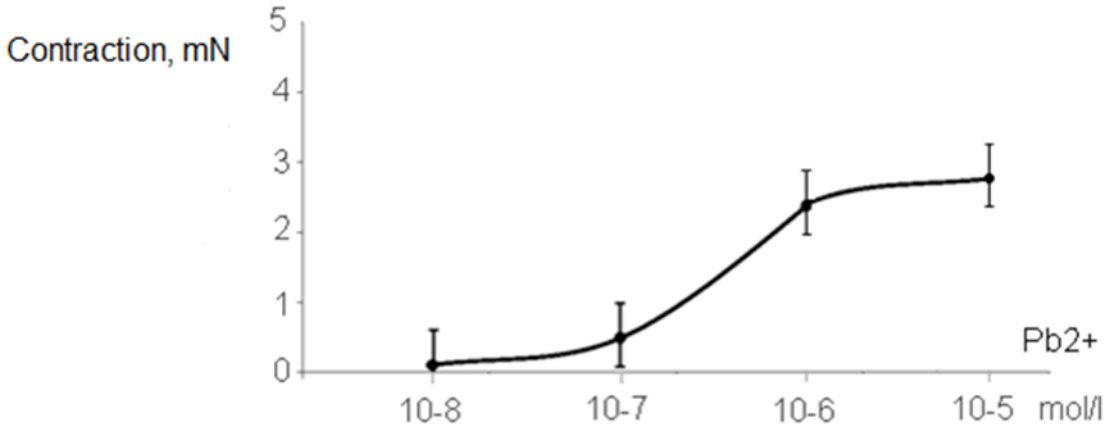

Fig.4. Contraction-concentration dependence of rat SM preparations of on gradually increasing Pb^{2+} concentrations (n=8).

The maxim contraction, achieved at $1x10^{-6}$ mol/l Pb^{2+} concentration, has an average measured force of 2.56 ± 0.44 mN. In the presence of $3x10^{-5}$ mol/l indomethacin (45 - 50 min) the force is significantly reduced (Fig. 5; n=9).

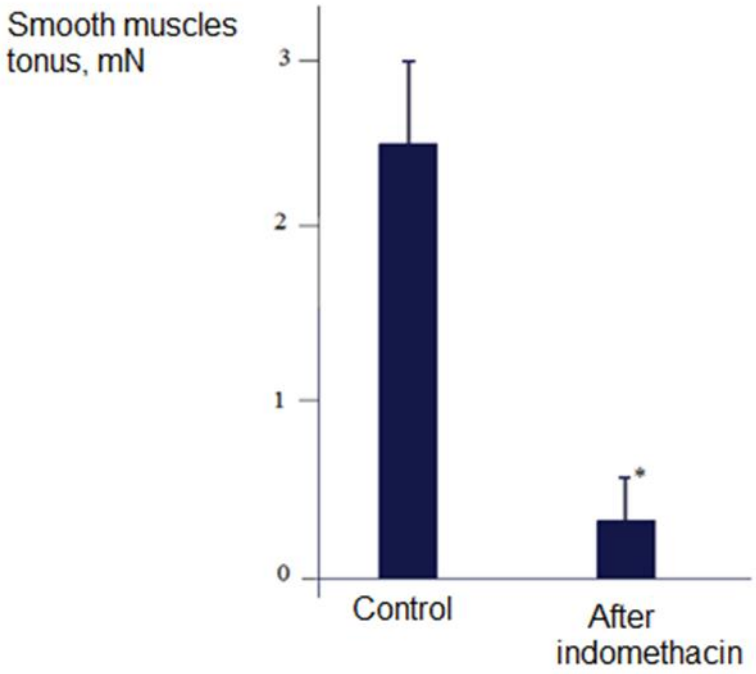

Fig. 5. Effect of indomethacin on lead-induced contractile activity of 1.10^{-6} mol/l Pb^{2+}, - $P < 0.05$.*

Similar results are achieved when the SM preparations (n=7) are treated with Acetysal – the contractions are significantly reduced, but not completely suppressed (Fig 6).

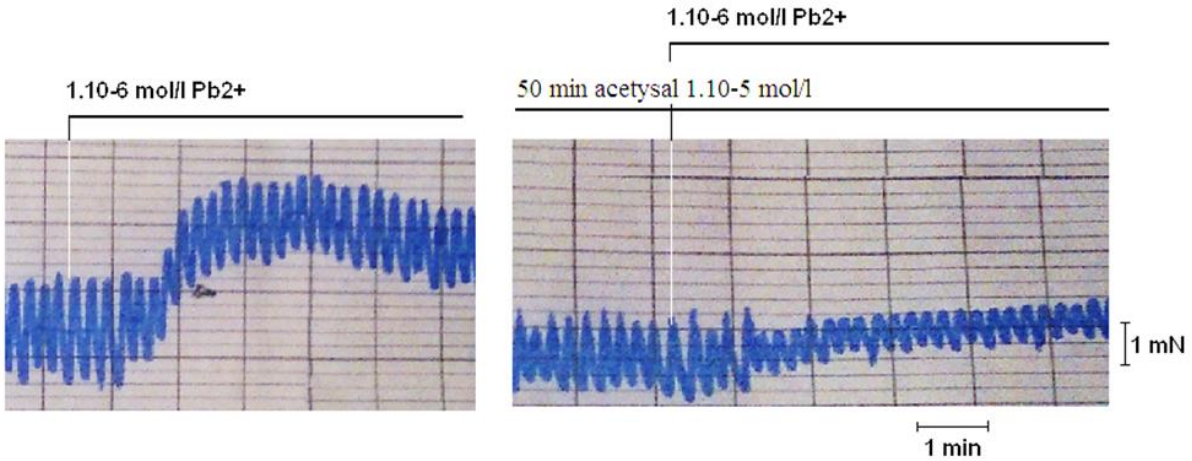

Fig. 6. Effect of acetysa on the contractile activity induced by 1.10^{-6} mol/l Pb^{2+}.

Application of TTX in concentrations of $2x10^{-4}$mol/l did not cause significant changes in the contractile activity of the SM preparations (n=5). Pre-treatment of SM preparations with TTX did not affect noticeably the magnitude of the $1x10^{-6}$mol/l Pb^{2+}–induced contraction (Fig.7).

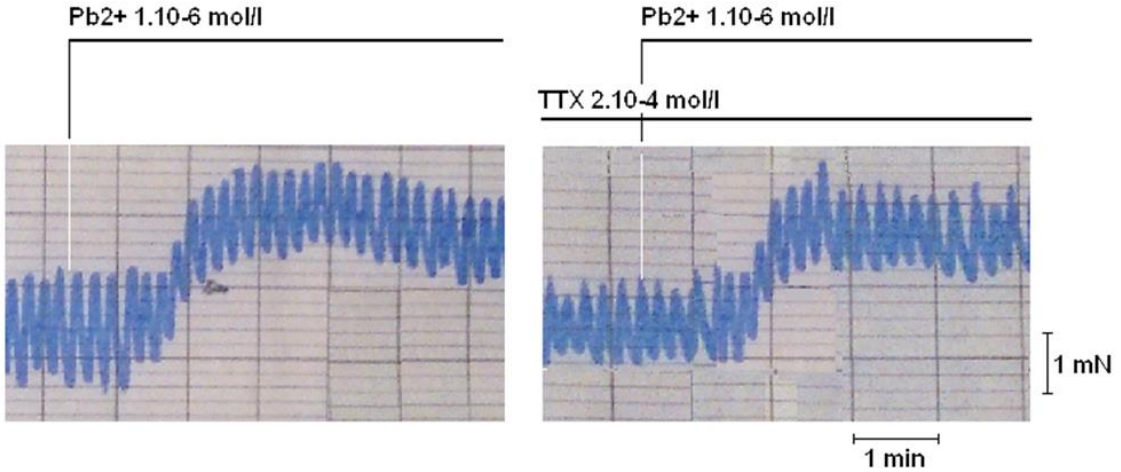

Fig. 7. Contractile reactions induced by 1.10^{-6} mol/l Pb^{2+}, without (a) and with TTX pre-treatment(b).

Table 2 summarizes the results of tests where the maximum amplitude of the Pb^{2+}-induced contractions in SM preparations are compared against the reactions of pre-treated SM preparations with receptor blockers: Atropine(1x10^{-6} mol/l), Hexametonium(1x10^{-5} mol/l), Raclopride (R-121; 1x10^{-5} mol/l), Methysergide (3x10^{-5} mol/l), and Phentolamine. None of the applied blockers caused significant changes in Pb^{2+}-induced reactions.

Table 2. Comparison between SM contractions induced by 1.10^{-6} mol/l Pb^{2+}(control), and contractions of SM preparations pre-treated with known receptor blockers.

Substances tested	Alternations on the tonus, mN			
	Control (Pb2+-induced reactions)	With 2 corresponding substances	N	P-value
atropine	2.41 ± 0.62	1.95 ± 0.36	9	> 0.05
hexametonium	2.41 ± 0.62	2.38 ± 0.50	6	> 0.05
raclopride	2.08 ± 0.44	2.12 ± 0.62	6	> 0.05
methysergide	2.72 ± 0.58	2.41 ± 1.02	5	> 0.05
Phentolamine	2.22 ± 0.66	2.36 ± 0.71	6	> 0.05

Discussion

According to the radiographic data, the leading abnormality among patients with high lead levels is the elevated local or diffused tonus of the stomach and the intestines.

This condition is commonly accompanied by deep, segmenting (the stomach) peristaltic activity, with uniform frequency.

Our experiments show that, within the applied concentration range (1x10^{-7} – 1x10^{-5} mol/l), Pb^{2+} causes contractions in SM preparations from stomach of rat, in concentration-dependent manner. Lead acetate has similar effect on other types of SM tissue, isolated from various test animals [3]. No change has been detected in the frequency and amplitude of the spontaneous contractions, which are considered the driver of the GIT peristaltic activity.

It is fair to say that the observed effect is not of neuronal nature. This is evidenced by the preserved character and strength of the Pb^{2+}-induced contraction in the presence of TTX, which has been shown to block the function of the intramural neural paths in in-vitro experiments [5]. The absence of significant neuronal effect is confirmed from the lack of change when receptors of neurotransmitters, such as acetylcholine, epinephrine, 5-HT, and dopamine, were blocked [8]. Similar conclusions about the effect of Pb^{2+}on levels of 5-HT, and 5-HT_{2B} receptors were made by [10], after experiments with SM tissue from rat’s aorta.

Fundamentally, the effect is a result of the direct myogenic action of ions on the SM cells, and it is related to the influence of Pb^{2+} on the prostaglandin synthesis.

It is well known that prostaglandins are derivatives of arachidonic acid, and are synthesized in the cells as a response to different stimuli. The influence of Pb^{2+} on the levels of arachidonic acid and PGE_2 secretion, as a consequence of elevated expression COX-2, is reported by [7] and [4].

Arachidonic acid is transformed into prostaglandins in an intracellular enzyme cascade of reactions, in which the leading enzyme systems are the constitutively expressed cyclooxygenase (COX-1), and the inducible cyclooxygenase isoform (COX-2). The mechanism of action the used in our tests drugs indomethacin and acetysal is linked to inhibition of COX-activity [6, 11], which could be reversible or irreversible. Both drugs interact relatively slow with the active center of the enzyme, consequently the effects of Pb^{2+} on the SM tissue takes place 45 – 50 min after the admission of the blocker in the tissue bath, when sufficient inhibition of the enzyme activity is achieved.

When COX enzyme systems are inhibited, Pb^{2+}-induced contractions are significantly suppressed. This

is conformation that, the contractions are fundamentally due to actions of prostaglandins, which synthesis is stimulated by presence of Pb^{2+} in the SM cells. The fact that two very different in its mechanism of action enzyme inhibitors (indomethacin and acetysal), can similarly reduce the Pb^{2+}-induced SM reaction, excludes the possibility of a specific, non-related to the prostaglandin synthesis action of any of the two drugs.

It is likely that the elevated prostaglandin levels activate the protein kinase C enzyme system [1, 27], which leads to contraction of the stomach SM tissue.

Conclusion

The correlation between in vitro results and the radiographic observations of functional disorders of GIT allow for the conclusion that Pb^{2+} affects intracellular contractile mechanisms (synthesis of prostaglandins) via non-specific paths, and therefore causes persistent changes in the intestinal motility.

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