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The Effect of Aluminum Injection in Lateral Ventricle on Sex Hormones in Male Rat.

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Abstract:

Aluminum is an important voltage sensitive calcium channels blocker and enter the body from different sources. This ion interferes with biological function of calcium ion. Because GnRH synthesis and secretion from brain nucleus dependent on calcium ion, this experiment was performed to study the effect of aluminum on male rat's sex hormones. The experiment was performed on foure groups of male rats, that the lateral ventricle were cannulated by sterotaxic surgery. Test group received 5.5 µmol ACSF containing 4.125 Pmol Aluminum in lateral ventricle for 20 days. Two series

of these animals after cannulation received the same volume of ACSF with pH=7.2 and 3.4. The shame control animals did not received any agent after cannulation. At the end of experiment, animals were anesthetised with nesdonal (sodium thiopental) over dose and sacrified and blood samples were collected the vas deferense, epididymis and testis were removed and weighted. Epididymis and vas deferense were dissected, cut and diluted with normal saline. Spermatozoid was counted by hemocytometer and count was justified per gram of tissues. The sex hormones were measured by RI method. Statistic test was student t-test and the results are expressed as mean ± SE and P <0.05 were significant. Results show that sex hormone and spermatozoid concentration per gram of tissues in vas deferense, epididymis and testis weight in the group which received Aluminum in lateral ventricle decreased significantly compared with shame control. These result indicated that Aluminum injection in rat's lateral ventricle can affect on sex hormones and Spermatozoid concentration per gram of these tissues and the weight of these organs , testis and the weight of these animals. Further studies will probably show the exact mechanism of Aluminum ion on sex hormone.

Key Words: Aluminum, Lateral ventricle, Epididymis, Vas deferense, Sex hormone.

Introduction:

Aluminum containers are widely used to cook, to freeze, or to wrap foods and it is known that Aluminum can migrate from containers to the food(1). This ion (Al3+) enter the body from many rout such as skin, lung, gastrointestinal tract and drugs(2,3,4,5). This ion can be accumulated in the body tissuse(5). Accumulation is

very high in the patient who has anemiae(7,18). Intraperitoneal(ip) administration of aluminum salts in rats, changed createnine clearance and urine concentration of bivalent ion such as Mg++ and Ca++ (8,9). Increases serum Aluminum causes disorder in enzymes reaction which has an element in its structures (10). Aluminum poisoning can inhibited signal transduction in cell membrane (11). The study show that Aluminum cause anemia in rat(12,13). Aluminum poisoning could affect on learning, memory and it is an important candidate for Alzheimer disease (14,15). Aluminum mine workers who have high level serum Aluminum, their TSH and prolactine significantly decreased compared with other workers (16). Renal failure patient which dialysis, and has high level serum Aluminum, show low reproductive power than others (17, 18). Aluminum is an important calcium channels blocker (19, 20). Voltage gated N-L-and T type calcium channels channels are blocked by Al3+ (21, 22). The studies show that Al3+ disrupts voltage gated Ca++ in synaptosomes(23). Zinc (Zn), Aluminum (Al), mercury (Hg) and leads (Pb) extracellulary applied, reduced voltage – activated calcium channels currents (VACCCs)(24). Intraventricular injection 5.5 μ mol Al for 5 days showed significantly decreased the long-term potential (LTP) in rats (25, 26). The study shows that Calcium ion is important for GnRH secretion in hypothalamus or other neurons which exist in other nucleuses in brain and effect on GnRH secretion (27). Because GnRH synthesis, secretion from brain nucleuse dependent on calcium ion, this experiment was performed to show the effect of Aluminum microinjection in rat's lateral ventricle (ICV) on sex hormone and reproductive system (vas deferense, epididymis, testis, and Spermatozoid concentration in these organs).

Materials and Methods:

Male Sprague –Dawley, albino rats weighing 235-347 gram (Razi institute Tehran, Iran), were housed in group cages under conditions of controlled (temperature 22-28ºC and illumination 12 h. Light cycle starting at 06 h minutes for least 10 days

before the experiments. Food and water were continuously available. Experiment were performed in (n=55) rats deprived of food for 24 h but given free access to water up to the beginning of the study. Rats were weight by Germany digital BA, 400, S, Sartorious weight (first weight) and anaesthetized with ip (Gedeon Richcer chemical works) ketamin 150 mg/kg. Each animal was implanted (at sterotaxic surgery) with cannula in the lateral ventricle to deliver AlCl3 and ACSF with different pH(7.2 and 3.4). The cannulas consisted of 21-1/2 guge stainless stell. Prior to surgery, each animal were anesthetized with ketamin and then were placed in a (Narishige Japan) sterotaxic unit. Animals lateral ventricle was unilaterally implanted (Paxinose Atlas). (Incisor bar: -6 mm below the interaural; AP=+1.4 mm from bregma; DV=+3.4 mm from surface of the brain; ML=±2mm from midline) .The cannulae were fixed to the skull with two stainless steel screws and dental cement. Following surgery, the rats were allowed to recover for one week. After recovery period, animals divided in four group. Test group who received 5.5 µmol ACSF containing 4.125 Pmol Aluminum in lateral ventricle for 20 days. Two series of these animals after cannulation received the same volume of ACSF with pH=7.2 or pH=3.4(at the injection time all animals were conscious). The shame control animals did not received any agent after cannulation. At the end of experiment, animals anesthetized with sodium thiopental (sepia nesdonal) and then scarified blood sampling were collected. Vas deferens, epididymis and testis were removed and weighted. Vas deferens and epididymis were dissected, cut and diluted with normal saline. Spermatozoid were counted by hemocytometer and count were justified per gram of vas deferens and epididymis tissuse(28). Sex hormones were measured by RI method. The testis removed weighted and placed in bouan fixator for study. After removed these organs and collecting blood sample, the animals were killed. Their brains were removed and for check the position 0.5µl thionin injected in guide cannulae by Hamilton syringe. The skull was rapidly removed and the brain placed in Formalin(10%) solution for 24 hours. After postfixation, brains were sectioned. Only those animals which cannulation were positioned in the appropriate

location were used for data analysis=50). Statistic test was student t- test, results are expressed as mean±SE and P<0.05 were significant.

Results:

Results showed that sex hormones (FSH, LH and testosterne) in test group received Aluminum in ICV, respectively(510±100 µu/ml,1240±90 µu/ml and 0.34±0.09 ng/ml) significantly decreased compared with the same control group(790±100 µu/ml,1920±180 µu/ml and 1074±0.38 ng/ml, table1). Vas deferens weight (106.5±2.3 mg) and Spermatozoid concentration per gram of this organ tissues in test group (43.68±1.74 millions) significantly decreased compared with control group(120.23±2.15 and 73.3±2.81 millions, table 2). Epididymis weight (490.3±8.45 mg) and Spermatozoid concentration per gram of this organ tissues in test group (79.78±3.08 millions) were significantly decreased compared with control group ((568.9±13.25mg and 107.73 ± 3.29 millions, table3). These values didn't show any significantly different in other groups (recieved ACSF with pH= 7.2 or 3.4 in ICV). Testis weight in test group (1520±30mg) significantly decreased compared with control group(1720±30mg).This parameter didn't show any significantly different in two series that received only ACSF with pH=7.2 or 3.4 in ICV. In spite that the first weight in control (283.92 ±9.27gr) and test group(292±6.36gr) didn't show any significantly different, the final weight in test group (262.77gr) show significantly decreased compared with control group(304±5.85gr, table 4,). This value didn't show any significantly different in that groups which received ACSF in ICV.

Table 1: sex hormones (FSH,LH and testosterone) in control and those groups that received ACSF with different pH or AlCl3 in their ICV for 20 days. *=P<0.05

Group, Number	Laboratory test (mean±SE)		
	FSH(µu/ml)	LH((µu/ml)	Testestrone(ng/ml)
Control, n=13	790±100	1920±180	1.72±0.38
ACSF/pH=7.2 ,n=12	710±190	1710±230	1.07±0.22
ACSF/pH=3.4	870±160	1640±180	1.00±0.18

,n=12			
ACSF/AlCl32 ,n=13	510±190	1240±90	0.43±0.09 *

In statistic analysis all groups compared with control and the volume injected was 5.5 µmol .

Table 2: Vas deference weight and spermatozoid concentration per gram of this organ in control and those groups that received ACSF with different pH or AlCl3 in their ICV for 20 days.*=P<0.05

Group, Number	(mean±SE)	
	Weight (mg)	Sperm Count (M/cc)
Control, n=13	120.23±2.15	8.55±0.25
ACSF/pH=7.2 ,n=12	115.3±2.65	8.95±0.45
ACSF/pH=3.4 ,n=12	117.3±2.12	8.36±0.9
ACSF/AlCl32 ,n=13	106.5±2.3	4.68±0.18 *

In statestic analysis all groups compared with control and the volume injected was 5.5 µmol .

Table 3: Epididymis weight and spermatozoid concentration per gram of this organ in control and those groups that received ACSF with different pH or AlCl3 in their ICV for 20 days.*=P<0.05

Group, Number	(mean±SE)	
	Weight (mg)	Sperm Count (M/cc)
Control, n=13	568.9±13.25	61.45±1.78

ACSF/pH=7.2 ,n=12	550±12.4	59.9±1.65
ACSF/pH=3.4 ,n=12	559±14.1	58.45±1.15
ACSF/AlCl32 ,n=13	490.35±8.45	38.55±1.67 *

In statestic analysis all groups compared with control and the volume injected was 5.5 µmol .

Table 4: The first and second weight in control and those groups that received ACSF with different pH or AlCl3 in their ICV for 20 days.*=P<0.05

Group, Number	(mean±SE)	
	First Weight (g)	Second Weight (g)
Control, n=13	283.92±9.27	304±5.75
ACSF/pH=7.2 ,n=12	296±7.67	299.58±6.6
ACSF/pH=3.4 ,n=12	291±5.25	294.58±5.52
ACSF/AlCl32 ,n=13	292±6.63	262.77±6.11 *

In statestic analysis all groups compared with control and the volume injected was 5.5 µmol .

Discussion:

Over observation in this study showed, that Aluminum injection in rat's lateral ventricle, influences on sex hormone (FSH, LH and testosterone) spermatozoid concentration per gram of vasa deferens, epididymis tissues and the weight of this organs and testis weight in test group (received Aluminum in ICV for 20 days). In addition the weight of test group significantly decreased compared with control group. On the otherhand, these values didn't show any significantly different in two series that received only ACSF with different pH in their lateral ventricle. The effect of Aluminum injection in the lateral ventricle on reproductive system did not investigated. But the heavy metals effect on central nervous system (CNS) were investigated. Administration Al3+ and Pb2+ in rat's synaptosome culture showed that

neurotransmitter release was significantly decreased compared with control group(9). Aluiminium injection in rat hippocampus showed that , the rate of glutamate neurotransmitter release, significantly decreased compared with control group(26). Because calcium ion is important for GnRH secretion and synthesis, in this experiment, the effect's of Aluminum injection in lateral ventricle, probably blocked voltage sensitive calcium channels (VSCC) in cells that responsible GnRH synthesis and decreased calcium influx in this cells and decreased the GnRH secretion in this cells (in this study we could not measure the blood GnRH). This phenomena causes that in test group may be decreased gonadothropine hormones (FSH and LH) in pituitary. Following by decreased FSH and LH, the rate of testosterone significantly decreased compared with control group and the reproductive factors affects from this hormones. Oral Aluminum adminstration(29) during pregnancy period, showed that growth retardation, delayed ossification and malformations at doses that also lead to reduced maternal weight gain. In over study Aluminum ICV injection may be affect on starving center and decreased animal test appetite and the weight of the animals were significantly decreased. Aluminum may be can binds relatively strongly to native DNA in cells that responsible GnRH produce, and alteration on it's functions. In present study Aluminum ICV injection probably enter the cells that synthesis and secretion GnRH and affects on its functions to deceased this hormone secretion. After decrease this hormone, their functions on reproductive system affects from this disorder. The results in this study showed that, Aluminum injection, in lateral ventricle affects on reproductive system and alter it's functions. Further studies will probably show the exact mechanism of Aluminum ion on spermatogenesis.

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