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Regional Alterations in the Levels of Brain Biogenic Amines, Glutamate, GABA, and GAD Activity Due to Chronic Consumption of Inorganic Arsenic in Developing and Adult Rats

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Arsenic contamination is widely prevalent in soils and waters of various parts of the world (World Health Organization, 1981). Arsenic consumption in humans has been reported to lead to cancer of skin and respiratory tract, liver damage, dermatosis, sensory - motor polyneuropathy, hearing loss, EEG abnormalities and in extreme cases to hallucinations, disorientation and agitation (Beckett et al. 1986; Binder et al. 1987; Mazumder et al.1988; Pershagen, 1986). In spite of awareness of its hazardous effects on the central nervous system (CNS) there have been no studies so far reported about the effects of chronic consumption of arsenic,particularly in the developing age, on biogenic amine neurotransmitters viz. noradrenaline (NA), dopamine (DA) and serotonin (5-HT).Hence this study was conducted to elicit information on the effects of chronic consumption of arsenic throughout developing age on the levels of these amines in different regions of the brain and the extent of restitution to normal levels after a long period of stopping consumption. In addition, it was also aimed to assess changes in glutamic acid decarboxylase (GAD) activity and in levels of glutamate and gamma-aminobutyric acid(GABA) as these amino acids are not only involved in carbohydrate metabolism but also in neurotransmitter pools. For comparative understanding, effects of arsenic intake in adult groups were also studied.

MATERIALS AND METHODS

For study of effects on developing brain, Wistar rat pups of either sex were administered arsenic in daily doses of 5 mg/kg body weight (0.208% sodium arsenate solution in distilled water) by gastric intubation from second day after birth upto 60 days of age. Control animals had simillar handling but received equivalent amounts distilled water. At 60 days of age, exposure

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to arsenic was stopped and the animals were rehabilitated for 100 days i.e., upto 160 days of age. The body weights of arsenic group and of age matched control group were monitored regularly. After weaning at 21 days of age, the food intake was also monitored. Control groups with age and weight matched (within $\pm$ 15%) to the arsenic animals were achieved by increasing the litter size (over 12) in the preweaning age and restricting the food intake to match that of arsenic exposed animals in postweaning age. For study of effects on adult brain, animals of 90 days of age were exposed to 5 mg arsenic/kg body weight/day through the diet. Exposure was continued for 90 days i.e., upto 180 days of age. Body weights of these and of respective controls were monitored.

At 20, 60 and after rehabilitation at 160 days of age or at 180 days of age in case of adults, the animals were decapitated and the brains were quickly removed. The following seven regions were dissected out : motor cortex, striatum, nucleus accumbens, hippocampus, hypothalamus, cerebellum and brainstem. Each brain region was weighed and rapidly processed for the estimation of either biogenic amines, glutamate and GABA as described elsewhere (Shailesh Kumar and Desiraju, 1990). The GAD activity was estimated by the method of Sadasivudu and Murthy (1978). The concentrations of NA, DA and 5-HT were simultaneously estimated in each tissue sample and the levels were expressed as nanograms of the amine/g tissue (ng/g). The concentrations of glutamate and GABA were expressed as µmoles of amino acid/g tissue (µmoles/g). GAD activity was expressed as µmoles of GABA formed/g tissue/hr. Significances of effects were analyzed by Student's *t* test (two tailed).

RESULTS AND DISCUSSION

Eye opening was delayed in arsenic exposed developing animals by 1-2 days. Some animals developed hair loss patches at about 20 days of age, but these disappeared spontaneously later on. Both developing group and adult group of animals exposed to arsenic had decreased body and brain weights and food intake compared to controls. At 20 days of age the arsenic exposed developing animals (n8) had a mean body weight of 31.3 ± 2.3g whereas control group (n8) weighed 46.5 ± 2.1g. The brain weights also were lower in the arsenic animals (1.32 ± 0.04g) than in controls (1.54 ± 0.06g) at this age. The deficits continued at 60 days also, the body and brain weights of arsenic animals being 103.5 ± 3.3g and 1.49 ± 0.11g respectively, and those of controls 135.3 ± 4.3g and 1.71 ± 0.09g. Rehabilitation (discontinuation of arsenic from 60 days of age) did

not result in the recovery of these deficits even by 160 days of age, when the body and brain weights of arsenic group (n8) were 224.7±20.4g and 1.69±0.08g respectively and of control group (n8) 292.5±18.9g and 1.75±0.12g. The study on adult group (n7) of animals of 90 days of age that were allowed arsenic intake for 90 days and assessed at 180 days of age also showed reduced body and brain weights, their values being 215.2±9.2g and 1.71±0.05g respectively, as against the normal group values of 258.7±16.1g and 1.86±0.06g.

The following were the salient changes noted in the levels of amines (NA, DA, 5-HT) and amino acids (glutamate, GABA, GAD activity) in the seven regions examined. The study on the developing group of animals revealed that by 60 days of age, the amines were mostly affected in cerebellum and hypothalamus (Fig.1, A sets). The other five regions showed either transient, or no effects at 20 days of age. The study on the adult group revealed effects in brainstem also, in addition to cerebellum and hypothalamus (Fig.1,B sets). Additionally, striatum and hippocampus showed elevated 5-HT levels while there was a reduction of DA in striatum and NA in hippocampus (Table 3). Discontinuation of the arsenic intake by developing group after 60 days of age resulted in no recoveries from the effects even by 160 days of age in cerebellum, hypothalamus, motor cortex and hippocampus(Fig.1, Table 1).

Table 1. Summary of the effects of chronic intake of arsenic on brain monoamine neurotransmitters in the developing animals. ↑-levels increased,↓-decreased.

Brain Region	Transmitter affected	Recovered after rehabilitation	Persisted after rehabilitation
Cerebellum	NA↓ DA↓ 5-HT↓	-	NA↓ DA↑ 5-HT↓
Hypothalamus	DA↓ 5-HT↓	DA	5-HT↓
Brainstem	NA↑ DA↑	NA DA	-
Striatum	5-HT↑	5-HT	-
Nucleus accumbens	DA↓	DA	-
Motor cortex	DA↑	-	DA↑
Hippocampus	DA↓	-	DA↓

GAD activity and GABA levels in the developmental group were reduced in cerebellum, hypothalamus and brainstem, but these were recovered following rehabilitation (Table 2), with the exception of GAD of brainstem. Glutamate changes were not significant in any of the regions except in brainstem (Table 2). On the contrary, in the adult group arsenic intake caused

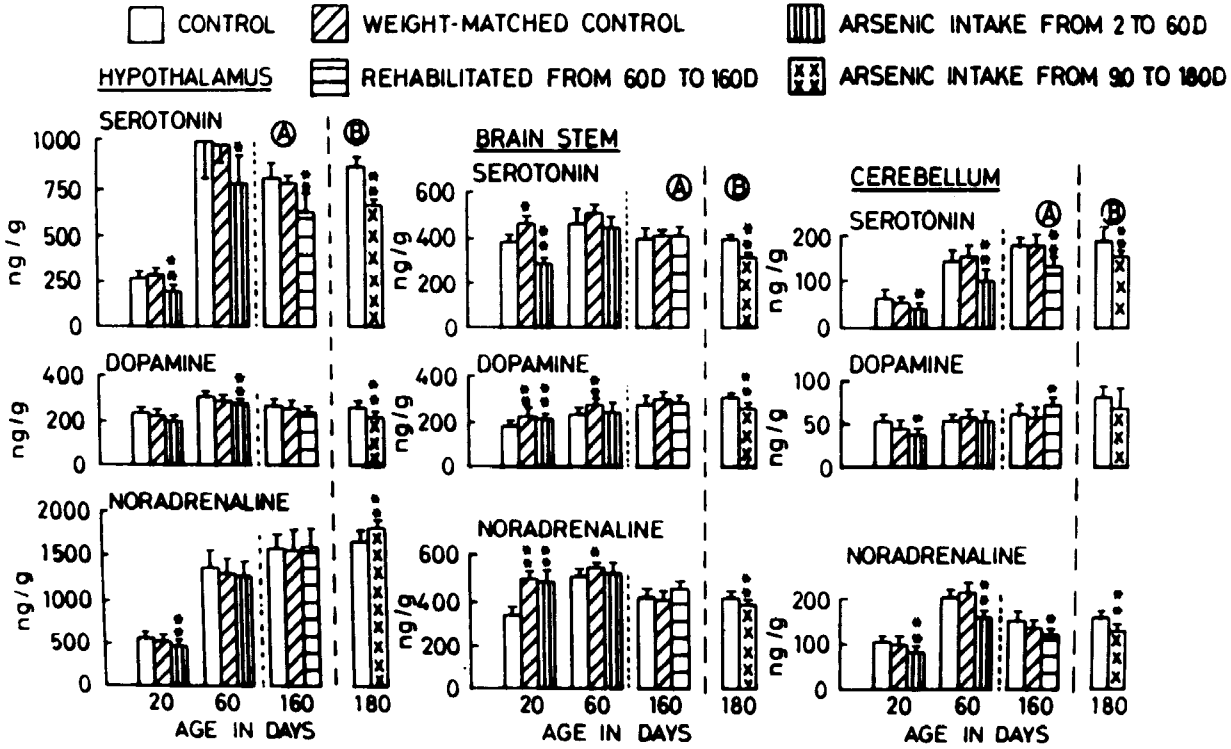

Figure 1. Effects of chronic intake of arsenic on brain NA, DA and 5-HT levels. Ⓐ sets of bars in each figure represent the developing group exposed to arsenic from 2 to 60 days of age and then rehabilitated upto 160 days of age. Ⓑ sets of bars represent the adult group exposed from 90 to 180 days of age. Values are expressed as mean ±S.D. of six experiments. * = $p<0.05$, ** = $p<0.01$.

Table 2: Effects of Chronic intake of arsenic on glutamate and GABA levels and on GAD activity. The developing group of rats were exposed to arsenic from 2nd postnatal day to 60 days of age and subsequently rehabilitated up to 160 days of age. The adult group was exposed to arsenic from 90 to 180 days of age. Glutamate and GABA levels are expressed as μmoles/g tissue and GAD activity as μmoles GABA formed/g/hr. Values are given as mean ±S.D of 6 rats. * = $P<0.05$; ** = $P<0.01$. C-normal control, WMC - weight matched control, As - exposed to arsenic as above, RH - rehabilitated group, AD - adult group.

	Age days	Cerebellum C	Cerebellum WMC	Cerebellum As	Hypothalamus C	Hypothalamus WMC	Hypothalamus As	Brainstem C	Brainstem WMC	Brainstem As
Glutamate	20	9.2±0.9	10.4±0.7*	9.4±0.6	7.3±1.6	8.6±0.5	7.5±0.6	5.7±0.7	6.4±0.3*	6.5±0.5*
	60	9.5±0.7	9.8±0.5	9.6±0.9	8.7±0.7	8.9±0.8	8.8±1.0	6.8±1.0	7.3±0.5	7.6±0.4*
	RH	9.6±1.1	9.6±0.9	9.7±0.6	8.1±0.7	7.9±0.7	7.1±0.4	6.3±0.9	6.3±0.7	7.5±0.5*
	AD	13.1±0.9	-	12.9±0.8	11.7±1.6	-	12.0±1.0	10.7±0.7	-	9.9±0.9
GABA	20	1.4±0.2	2.0±0.3**	1.5±0.2	2.5±0.7	2.4±0.5	1.6±0.3**	2.0±0.3	2.0±0.3**	1.7±0.3
	60	1.5±0.3	1.6±0.2	1.4±0.2	3.8±0.4	4.0±0.7	2.8±0.3**	1.8±0.4	2.4±0.2**	1.9±0.2
	RH	1.9±0.4	2.1±0.3	1.6±0.2**	4.4±0.4	4.3±0.3	4.4±0.2	2.3±0.6	2.3±0.6	2.2±0.4**
	AD	2.7±0.4	-	2.0±0.4**	6.7±0.6	-	6.3±0.9	2.2±0.4	-	1.6±0.2
GAD activity	20	11.9 ±1.8	11.5 ±2.1	10.7 ±1.3	16.7 ±2.1	14.6 ±1.8	14.1** ±1.1	6.6 ±2.6	4.1* ±1.8	5.9 ±1.7
	60	30.2 ±4.9	28.9 ±2.7	21.7** ±3.8	26.6 ±4.1	24.6 ±3.1	22.1* ±1.8	26.6 ±2.7	23.4* ±2.8	21.6** ±3.1
	RH	35.4 ±4.6	33.4 ±3.3	31.5 ±2.7	29.4 ±3.6	31.5 ±2.6	30.3 ±4.1	25.6 ±2.8	26.7 ±1.7	22.6* ±1.7
	AD	35.9 ±2.0	-	29.6** ±3.4	31.8 ±2.8	-	26.1** ±3.2	18.7 ±3.1	-	16.6 ±4.6

increase of glutamate levels in motor cortex, striatum, nucleus accumbens and hippocampus (Table 3). In these adults, GABA levels were reduced in striatum, cerebellum and brainstem, while GAD activity was reduced in nucleus accumbens, hippocampus, hypothalamus and cerebellum.

Table 3. Effects of chronic intake of arsenic on NA, DA, 5-HT, glutamate, GABA and GAD activity in adult group. Animals were exposed from 90 to 180 days of age. Amine levels are expressed as ng/g tissue, glutamate and GABA levels are expressed as μmoles/g tissue and GAD activity as μmoles GABA formed/g/hr. Each value is mean + S.D. of 6 rats. Rest of the legends are same as in Table 2.

Brain region	NA		DA		5-HT	
	C	As	C	As	C	As
Striatum	281.7 +15.4	261.4 +20.2	3918.7 +200.1	3610.1** +111.7	300.4 +43.4	357.8** +27.1
Hippo-campus	315.2 +11.5	275.1** +15.1	71.1 +16.1	68.1 +14.6	243.4 +15.7	272.5** +21.3

	Glutamate		GABA		GAD activity	
	C	As	C	As	C	As
Motor cortex	11.8 +0.69	14.3** +0.42	2.1 +0.4	2.1 +0.5	17.1 +2.1	17.2 +1.4
Striatum	16.23 +1.7	18.7** +0.8	4.5 +0.5	3.3** +0.6	31.6 +4.2	28.6 +3.8
Nucleus accumbens	14.4 +1.6	16.1* +1.2	3.6 +0.3	3.8 +0.4	17.9 +3.4	14.7* +2.0
Hippo-campus	15.5 +1.3	16.9* +0.7	2.9 +0.3	3.1 +0.4	18.9 +1.8	15.0* +0.9
Hypotha-lamus	11.7 +1.6	12.0 +0.9	6.7 +0.6	6.3 +0.9	31.8 +2.8	26.1** +3.2
Cerebellum	13.1 +0.9	12.9 +0.8	2.7 +0.4	2.0** +0.2	35.9 +2.0	29.6** +3.4
Brainstem	10.7 +0.7	9.9 +0.9	2.2 +0.4	1.6** +0.2	18.7 +3.1	16.9 +4.6

The study on developmental group showed that decreasing food intake of a control group (undernutrition) to a level that was consumed by arsenic group caused reductions in body and brain weights similar to those of arsenic group. Hence these weight changes of arsenic group are mainly due to lowered food intake. But the trends of changes (compared to respective normal control group values) occuring in the amines and amino acids were not similar in the arsenic group and the underweight (undernutrition) group (Fig.1, A sets

and Table 2). Generally, the underweight group showed changes in a trend of elevations, in contrast to the trends of decreases in the arsenic group with only a few exceptions.

Arsenic can pass through blood-brain barrier. It can inhibit mitochondrial respiration and carbohydrate metabolism (Ghafgazi et al. 1980). It can also accumulate in the brain when animals are exposed continuously and can influence ATP metabolism (Valkonen et al. 1983). Hence arsenic could significantly influence neurons and glia. This study on rehabilitation effects on the developmental group revealed no recovery in amine levels of some regions. Since arsenic has a half-life of 60 days in rats (World Health Organization, 1981), the cumulating effects of continuous intake of arsenic may have culminated in inducing the reorganization in developing brain, and the subsequent rehabilitation could not have succeeded in reversing some of the altered expressions of neuropil development.

There are no previous reports on the effects of chronic consumption of arsenic on either the levels of monoamines or on the enzymes regulating their metabolism. But there are reports that arsenate can act as a phosphate analog and can inhibit phosphate dependent reactions (Alves and de Meis, 1987). Hence the pyridoxal 5'-phosphate dependent reactions in monoamine metabolism : i) conversion of L-Dopa to DA and ii) conversion of 5-Hydroxytryptophan to 5-HT may be affected. Arsenate can competitively inhibit these reactions, thereby leading to reduced levels of DA, NA and 5-HT. Inorganic arsenic can also bind to sulfhydryl groups of enzymes and inhibit their activity.

Glutamate and GABA estimations made in this study include both metabolic and transmitter pools. But, as GAD activity is mainly linked to maintenance of the neurotransmitter pool of GABA, any reduction in its activity may lead to reductions in the transmitter pools of GABA, which is a major inhibitory transmitter in the brain. GAD activity was inhibited in some regions. It is plausible that reductions in GAD activity might be due to arsenate mediated enzyme inhibition since GAD requires pyridoxal 5'- phosphate as a co-factor. The increase in glutamate content would have been mostly in the metabolic pool as no serious excitotoxic effects were found in these animals.

Arsenic toxicity is generally known to result in encephalopathy and psychopathological responses (Beckett et al. 1986; World Health Organization, 1981).

It is significant to note that although the present results revealed that cerebellum, hypothalamus and brainstem undergo changes in the amines and amino acids more than the other regions, the arsenic rats showed no overt morbidity or abnormalities in behavior. Only when tested in operant conditioning with food reward in continuous reinforcement schedule or in fixed ratio-5 schedule, there was a significant increase in the time required for learning (*our unpublished observations*). Hence, the effects produced by arsenic may depend on age, dose and route of intake and the present study revealed a latent, minimal brain dysfunction of a sub-toxic level and the changes in transmitter levels evoked in the system may mostly be of adaptive kind for homeostatic adjustment.

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