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Effects of Methyl Mercury, Mercuric Sulfide and Cinnabar on Active Avoidance Responses, Na^+/K^+-ATPase Activities and Tissue Mercury Contents in Rats

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ABSTRACT

This study compared the neurobehavioral toxicities of three mercurial compounds: methyl mercury (MeHg) which is soluble and organic, and mercuric sulfide (HgS) and cinnabar (naturally occurring HgS), which are insoluble and inorganic. Cinnabar, a Chinese mineral medicine, is still used as a sedative in some Asian countries, but there is relatively little toxicological information about it. These mercurial compounds were administered intraperitoneally (MeHg, 2 mg/kg) or orally (HgS and cinnabar, 1.0 g/kg) to male rats once every day for 13 consecutive days with assays conducted during or after discontinuous administration for 1 h, 2, 8 and 33 weeks. Neurotoxicity was assessed based on the active avoid-ance response and locomotor activity. The results obtained showed that MeHg and cinnabar prominently and irreversibly caused a decrease in body weight, prolongation of latency for escape from electric shock, a decrease in the percentage for the conditioned avoidance response (CAR) to electric shock, impairment of spontaneous locomotion and inhibition of Na^+/K^+-ATPase activity of the cerebral cortex. In contrast, HgS reversibly inhibited spontaneous locomotion and Na^+/K^+-ATPase activity. It was noted that HgS significantly decreased the latency of escape from electric shock during the ad-ministration period, which lasted for 33 weeks after discontinuous administration. In fact that pretreatment with arecoline (a cholinergic receptor agonist) but not fipexide (a dopaminergic receptor agonist) could significantly shorten the prolonged latency for escape caused by MeHg and cinnabar, suggested that the deficit in the active avoidance response was perhaps, at least in part, mediated by the dysfunction of the cholinergic rather than the dopaminergic system. Determination of the Hg levels of the whole blood and cerebral cortex revealed that the tissue mercury content was highly correlated with the degree of neurobehavioral toxicity of these Hg compounds. These findings suggest that insoluble HgS and cinnabar can be absorbed from the G-I tract and distributed to the brain. The possibility that contamination due to other minerals in the cinnabar is responsible for the greater neurotoxic effects compared to HgS is under investigation.

Key Words: HgS, cinnabar, active avoidance response, locomotion, tissue mercury contents, Na^+/K^+-ATPase

I. Introduction

In recent years, more and more attention has been paid to the possible adverse health effects of mercury exposure due to industrial use of methyl mercury (MeHg) and its analogues (Pedersen *et al*., 1999; Santucci *et al*., 1998). Recently, some reports have indicated that the mercury-soil pollution (mostly mercuric sulfide) is cumulative as a consequence of the enormous increase in contaminant mercuric compounds in Asia (Zetterstrom, 1999). However, a naturally occurring inorganic mercuric sulfide (HgS) is an ingredient in some Chinese traditional medicines, *e.g*., cinnabar, which has been used in Chinese herbal medicine as a memory-enhancing drug for more than 2000 years (Kang and Oransky, 1992). There is no doubt that its significant accumulation in tissues and organs might produce neurological dysfunction, *e.g*., a decrease in intellectual performance, including short-term memory, cognitive abilities and spatial learning (Bowie *et al.*, 1998; Hua *et al*., 1996; Gilbert and Grant-Webster, 1995). Whether HgS and cinnabar administration can influence learning acquisition, memory retention or subsequent intoxication, as evaluated based on behavioural and neurochemical changes, is worth studying.

Recent researches have indicated that MeHg treatment

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resulted in widespread cortical and cerebellar alterations, characterized by reduced myelination, delayed migration and loss of neurons. The morphological alterations were accompanied by permanent alterations in learning and memory as well as altered pharmacological sensitivity in catecholaminergic systems (Annau and Cuomo, 1988). Meanwhile, MeHg could interfere with cholinergic neurotransmission by affecting the regulatory step in acetylcholine synthesis and by increasing the spontaneous release of transmitter (Levesque *et al*., 1992). Fipexide (a dopaminergic receptor agonist and a parachlorophenossiacetic acid derivative) and arecoline (a muscarinic receptor agonist) were able to improve acquisition or retrieval performance in active avoidance tests, when they were administered just before training or trial testing (Genkova-Papazova and Lazarova-Bakarova, 1996; Raffaele *et al*., 1996). On the basis of this finding, we tried to test whether treatment with either fipexide or arecoline would result in improvement of the altered active avoidance response when used in combination with mercurial compounds during 13 consecutive days of training. In addition, a previous report indicated that amnesia induced by the Na^+/K^+-ATPase inhibitor, ouabain, produced a retrieval deficit and loss in long-term memory storage (Gibbs and Ng, 1979). Recent neurochemical data further showed that aluminum exposure also produced significant deficits in acquisition and retention of learned response in active avoidance situations via a significant decrease in the Na^+/K^+-ATPase activity of the brain in rats (Lal *et al*., 1993). Therefore, we also studied whether these mercurial compounds administered to rats also altered the Na^+/K^+-ATPase activity of the cerebral cortex.

Although HgS is regarded as inorganic and insoluble, it is important to know whether it can be absorbed in the gastrointestinal tract and then distributed to the brain, thus producing neurobehavioral toxicity. In this study, we compared critical brain mercury concentrations in association with specific neurobehavioral and concurrent neurobiochemical changes, including active avoidance responses, spontaneous locomotion and neuronal Na^+/K^+-ATPase activity, which are considered to be indicators of neurobehavioural deficits in rats. Daily feeding with high doses (1.0 g/kg) of HgS and cinnabar, which were regarded as over- or abused- dosages as in the reported cinnabar intoxication cases (Prakash *et al*., 1995) was carried out. We believe that an understanding of the neurobehavioral and concurrent neurochemical toxicities of HgS and cinnabar is ugently needed. This was also important because we sought to establish legislative regulation and management of clinical intoxication cases.

II. Materials and Methods

1. Animal Preparations

Randomly bred adult male Sprague-Dawley rats N = 10 per cage, were housed at 23 ± 2°C with food and water ad lib in all the experiments. They were maintained on a 12:12 light: dark cycle, with all tests conducted during the light phase of the cycle. The study was conducted in accordance with the guidelines for the care and use of Laboratory Animals set by the Animal Research Committee of National Taiwan University, College of Medicine. Rats weighing 180 – 200 g were administered daily by either orally dosing with commerical cinnabar (1.0 g/Kg) and mercuric sulfide (HgS; 1.0 g/Kg) suspended in normal saline, or by intraperitoneally injecting with methyl mercury (MeHg; 2.0 mg/Kg) dissolved in normal saline or saline alone (control vehicle) for 13 consecutive days. By means of inductively coupled plasma (ICP)-MS analysis, we selected cinnabar (mercury: 855.1 mg/g) and mercuric sulfide (mercury: 862.1 mg/g) for our experiments. In order to understand whether the mercurial compounds have analgesic effect, we conducted tail flick testing by placing the rat's tail over a radiant heat source (50 W projector lamp). After dosing for 13 consecutive days, neither cinnabar nor HgS induced an inhibitory effect on the thermal induced hyperalgesia throughout 30-min intervals. However, MeHg exerted a reversible antinociceptive effect, which completely diminished after discontinuous administration for 2 weeks (data not shown). In the first active avoidance task, mercurials were administered 1h prior to training once every day, and then the latency for escape and the percentages of conditioned avoidance response (% CAR) were measured for 13 consecutive days. After discontinuous administration for 8 and 33 weeks (without continuous training), the latency for escape and % CAR were also tested for memory retention (long-term memory). In the second active avoidance task, a single oral dose of fipexide (10 mg/Kg) or arecoline (4.0 mg/Kg, intraperitoneal injection) was administrated 15 mins before testing to those rats which had been trained for 13 consecutive days as described above on the 14th day. The latency for escape and % CAR were measured to evaluate the learning acquisition. In addition, changes in body weight (% of control before treatment) were monitored throughout the experimental period. These animals were sacrificed under deep anesthesia with pentobarbital (60 mg/Kg, IP) at times varying from 2 weeks to 33 weeks after was discontinued treatment. The whole blood, liver, kidney and cerebral cortex were removed. Tissue homogenization and whole blood were used for detection of mercury. In addition, the cerebral cortex of the rats were assayed for Na^+K^+-ATPase activity (Gerbi and Maixent, 1999).

2. Two-Way Active Avoidance Task

Animal behavior in terms of active avoidance responses was measured using of a shuttle box (Ugo Basile Automatic Reflex Conditioner Cat. model 7530). The instrument, measuring 21 × 48 × 22 cm, was divided into two equal sized chambers, separated by a barrier (3 inches high) and divided by a metal partition in the middle with a circular opening in

the centre that was large enough for the rat to pass through. The apparatus had a stainless-steel grid floor, and the chambers were separated by a slit door. The grid floor was wired via a transfer relay to permit a single shocker's output to be switched to either side of the cage. Learning sessions consisted of placing animals into a two-compartment cage, where they learned to escape to the safe compartment (an open door) after an ultrasonic tone and light anticipating an electric foot shock. The cages are provided with acoustic (40 dB) and visual (30 W) stimulators, which supplied conditioning stimuli. The unconditioned stimulus (UCS: a scrambled foot shock) started 10 s after onset of the conditioned stimuli (CS: a tone and a light) if the rat had not made an avoidance response by moving to the other compartment during CS presentation. Both stimuli were terminated together when the rat escaped to the other compartment or when 10 s had elapsed, whichever event occurred first. The animals received 20 trials per day during both the acquisition (13 consecutive days) and retention (2 – 33 weeks later) session. The behaviours recorded were the latency to cross, measured based on CS onset, and a CAR based on the number of inter-trial crossings (the number of times the rats crossed from one half of the box to the other between trials, i.e., when neither conditioning stimuli nor shock was being delivered). As these procedures were repeated, the risk and its avoidance were learned through learning acquisition and memory retention (Hogg *et al*., 1998).

3. Open Field Testing

To test for animal habituation (novel conditions), the rats were placed in an open field before, or 1 day and 14 days after the last administration. A large white rectangular box was used (70-cm wide, 90-cm long and 60-cm high) that a total of 63 square $(10 \times 10\ \text{cm})^2$ were drawn with black lines on the white floor of the field. The presence of six events was quantified by an automated image analysis system during an observation period of 60 min: squares crossed, rearings, grooming bouts, as previously described (Page and Terry, 1997). The number of squares crossed and the number of rearings were counted during a period of 60 min, and the activity boxes were localized in dark cabinets in a quiet room.

4. Na^+/K^+-ATPase Activity Assay

Membrance ATPase activities were assayed (Liu *et al*., 1997; Rohn *et al*., 1993). The method allowed quantification of two distinct Na^+/K^+-ATPase and Mg^{2+}-ATPase activities in the same sample. The enzymatic activities were measured in quadruplicate in covered 96 well microtiter plates at 37 ± 0.5°C on a shaker. Ninety microliters of assay buffer (10% sucrose pH = 7.2) containing 2 μg of membrance protein were added to each well. The Na^+/K^+-ATPase activity was determined by subtracting the ouabain (3.74 mM) insensitive activity from the overall $Na^+/K^+/Mg^{2+}$-ATPase activity. The plate was preincubated at 37 ± 0.5°C for 20 min, and the assay was started by adding of 10 μl of ATP (final concentration 5 mM) making a final reaction volume of 100 μl. The reaction was terminated by adding of 200 μl of malachite green (MG) plus ammonium molybdate (AM) (3:1). The plates were read on a microplate ELISA reader (MR7000, Dynatech, U.S.A.) at 630 nm. The absorbance values obtained were converted to activity values by means of linear regression using a standard curve of sodium monobasic phosphate included in the assay procedure. Released Pi (μmole) represents the concentration of inorganic phosphate released by the enzymatic hydrolysis of ATP, which was determined colorimetrically. Finally, The ATPase activities were expressed as micromoles of inorganic phosphate per milligram protein per 20 minutes. Values reported represent the mean and SEM of at least three separate experiments.

5. Determination of Mercury Content

One ml of blood and 200 mg of liver, kidney or cerebral cortex tissues were placed in a wide-mouth linear polythylene scintillation vial, and 3 ml of a 3:1 mixture of concentrated nitric acid: 70% perchloric acids were added along with 50 mg of vanadium pentoxide. The vials were capped and allowed to stand overnight at room temperature. Following predigestion, the capped vials were heated for 4 hr on a shaking water bath at 68 ± 0.5°C, then uncapped and heated again for 3 hr. After cooling, five drops of 30% hydrogenperoxide were added and the vials were again capped and allowed to stand overnight to complete the digestion process. Suitable dilutions were made from the digested material, and the total mercury content was determined with a solution of 2% $SnCl_2$ in 0.05 M H_2SO_4. In order to analyze total tissue Hg content, cold-vapor atomic absorption spectrophotometry (AAS) was performed using automated equipment. The reducing agents and apparatus were the same as those reported previously except that 1.0 ml of diluted digest was placed in a modified Erlenmeyer flask, and then 4.0 ml of reducing mixture were added to the closed system through a flexible plastic tube sealed into the ball-joint cap and connected to a syringe type automatic pipette (Iverson *et al*., 1974). Finally, the mercury content in the liver, kidney and blood of rats was measured by means of cold vapor AAS.

6. Statistical Analysis

Results are expressed as mean ± SEM. Analyses of variance (ANOVA) were used to evaluate the results. When the F value was significant, the significance between groups was assessed by means of Dunnett's t-tests for comparison between group means as described previously (Ndamba *et al*., 1997). It was considered to be significant when the p

value was less than 0.05.

III. Results

1. Effects of Mercurial Compounds on Body Weight

It was found that MeHg (2 mg/Kg) and cinnabar (1.0 g/Kg) significantly decreased the body weight of the treated-rats ($F = 6.47$ and 6.29, respectively, $p < 0.05$), but that HgS (1.0 g/Kg) did not produce such an effect ($F = 1.84$, $p > 0.05$) as compared to the normal control rats (Fig. 1).

2. Effects of Mercurial Compounds on Active Avoidance Response

The active avoidance responses was represented by the averaged latency of escape (20 trials). Following administration for 13 consecutive days, we found that MeHg as well as cinnabar, ($F = 13.2$ and 12.2, respectively, $p < 0.05$) but not HgS, treatment impaired escape performance as revealed by a significant increase in the latency of escape response. In contrast, Fig. 2(a) shows that HgS could decrease the latency of escape starting at day 5 and continuing until day 13 ($F = 5.84$, $p < 0.05$). After administration was discontinued 2 to 33 weeks, both MeHg and cinnabar had an impairing effect on the ability to escape for electrical shock ($F = 14.2$ and 13.1, respectively, $p < 0.05$). Interestingly, rats treated with HgS showed prolonged retention until 33 weeks as compared with

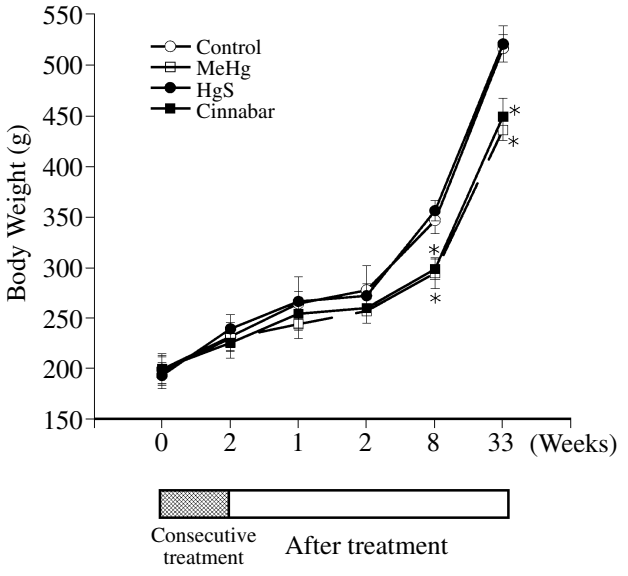

Fig. 1. Effects of mercurial compounds on the body weight of rats. Ten rats in each group were administered with either MeHg (2.0 mg/Kg, IP), HgS (1.0 g/Kg, PO) or cinnabar (1.0 g/Kg, PO), respectively, once every day for 13 consecutive days. The body weights were continuously recorded during and after treatment with mercurial compounds. Data are presented as mean ± SEM.

*$p < 0.05$ as compared with control.

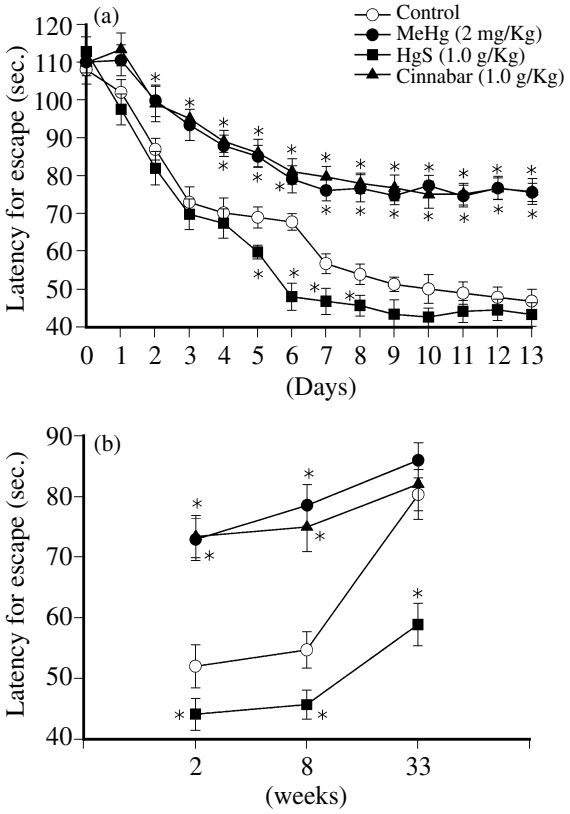

Fig. 2. Effects of mercurial compounds on learning acquisition and memory retention in rats. Ten rats per group were administered with either MeHg (2.0 mg/Kg, IP), HgS (1.0 g/Kg, PO) or cinnabar (1.0 g/Kg, PO), respectively, for 13 consecutive days. The latency (sec) for escape was recorded and calculated during 13 consecutive days (a) and at 2, 8 and 33 weeks after discontinuous treatment (b). Data are presented as mean ± SEM.

*$p < 0.05$ as compared with control.

the vehicle controls (Fig. 2(b), $F = 9.1$, $p < 0.05$). From the CAR tests, our data indicated that MeHg as well as cinnabar lowered the percentage of CAR ($F = 11.8$ and 12.5, respectively, $p < 0.05$) while HgS produced a slight increase on the percentage of CAR ($F = 2.9$, $p > 0.05$) during 13 consecutive days (Fig. 3(a)) and also during various periods (Fig. 3(b), $F = 5.31$, $p < 0.05$) after administration was discontinued as compared with the vehicle controls. Furthermore, we found that the deficit in memory retention judged based on electrical avoidance induced by either MeHg or cinnabar administration for 13 consecutive days, could be partially reversed by pretreatment with arecoline (Fig. 4(b) as compared with the control, $F = 1.4$ and 1.7, respectively, $p > 0.05$) but not by fipexide (Fig. 4(a) as compared with the control, $F = 12.8$ and 13.0, respectively, $p < 0.05$).

3. Spontaneous Locomotion of MeHg and HgS in the Open Field

Spontaneous locomotion is used as a biomarker for de-

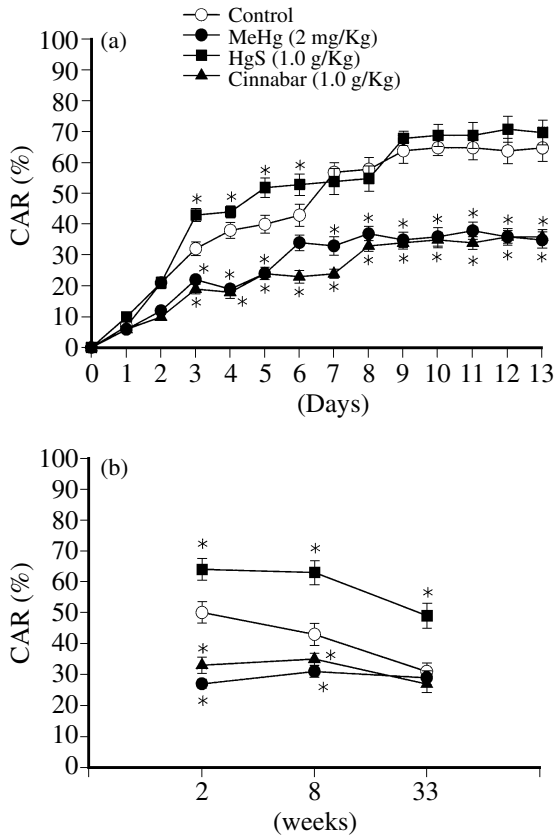

Fig. 3. Effects of mercurial compounds on the conditioned avoidance response (CAR) of rats. Ten rats per group were administered with either MeHg (2.0 mg/Kg, IP), HgS (1.0 g/Kg, PO) or cinnabar (1.0 g/Kg, PO), respectively, for 13 consecutive days. In 20 trials, the number of correct for escaping electroshock was recorded and calculated during 13 consecutive days (a) and at 2, 8 and 33 weeks after discontinuous treatment (b). Data are presented as mean ± SEM.
*$p < 0.05$ as compared with control.

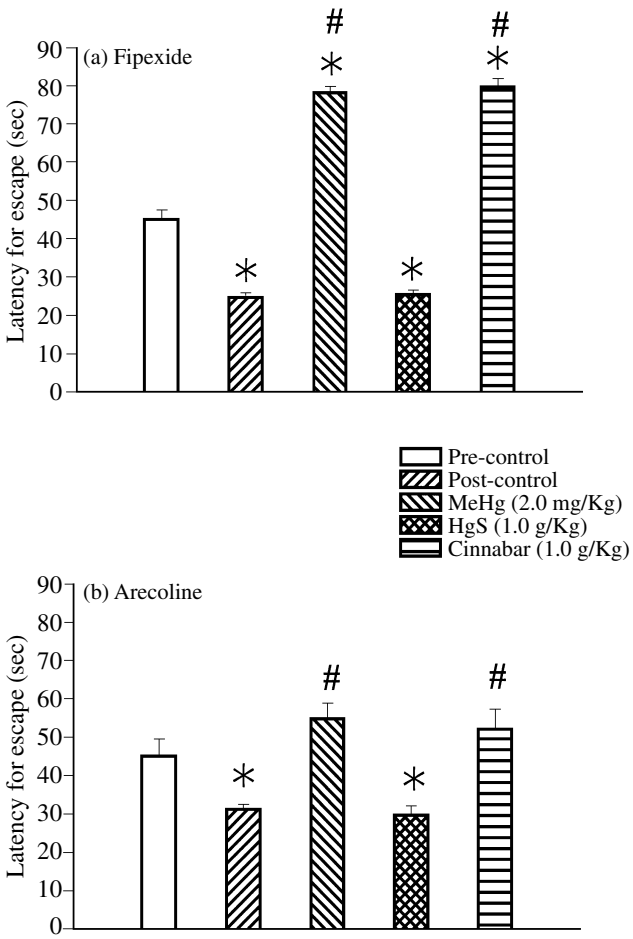

Fig. 4. Effects of mercurial compounds on avoidance learning acquisition in rats. Ten rats per group were administered with either MeHg (2.0 mg/Kg, IP), HgS (1.0 g/Kg, PO) or cinnabar (1.0 g/Kg, PO), respectively, for 13 consecutive days. After discontinuous treatment for 24 h, all of the tested rats treated with mercurial compounds were administered with fipexide (a: 10 mg/Kg, PO) or arecoline (b: 4.0 mg/Kg, IP), respectively, 15 mins prior to recording of the avoidance response and the latency. Data are presented as mean ± SEM.
*$p < 0.05$ as compared with control.

tecting the central function of neurotransmission. Dosing with MeHg or cinnabar once every day for 13 days clearly produced both sedation or hypoactivity at 1 h (Fig. 5(b), F = 12.4 and 10.9, respectively, $p < 0.05$) and 2 weeks (Fig. 5(c), F = 8.1 and 7.3, respectively, $p < 0.05$) after the last administration. Meanwhile, HgS only caused hypoactivity at 1 h (Fig. 5(b), $F = 6.5$, $p < 0.05$) after treatment was discontinued, whereas all the treated groups as well as the control group had almost the same locomotion in the open field prior to the first treatment (Fig. 5(a)).

4. Effects of MeHg, HgS and Cinnabar on Na^+/K^+-ATPase Activity

In the enzymatic assay of Na^+/K^+-ATPase activity on the cerebral cortex, it was found that MeHg as well as cinnabar significantly and irreversibly inhibited Na^+/K^+-ATPase activity (F = 9.2 and 9.4, respectively, $p < 0.05$). However, the HgS-treated group only reversibly reduced this enzymatic activity ($F = 6.4$, $p < 0.05$) 24 h after the last administration (Fig. 6). While 8 weeks after this treatment was discontinued, either MeHg or cinnabar still produced 50% inhibition of this Na^+/K^+-ATPase activity (F = 6.9 and 6.7, respectively, $p <$ 0.05).

5. Tissue Mercury Contents after MeHg and HgS Administration

Using of cold vapor AAS, we determined the mercury contents in various tissues (whole blood, liver, kidney and cerebral cortex) of rats after daily dosing for 13 days with MeHg and HgS. Figure 7(a) shows that mercury could largely accumulate in blood, liver and kidney, reaching 1.2, 1.5 and 7.0 ppm respectively after dosing for 13 consecutive days with

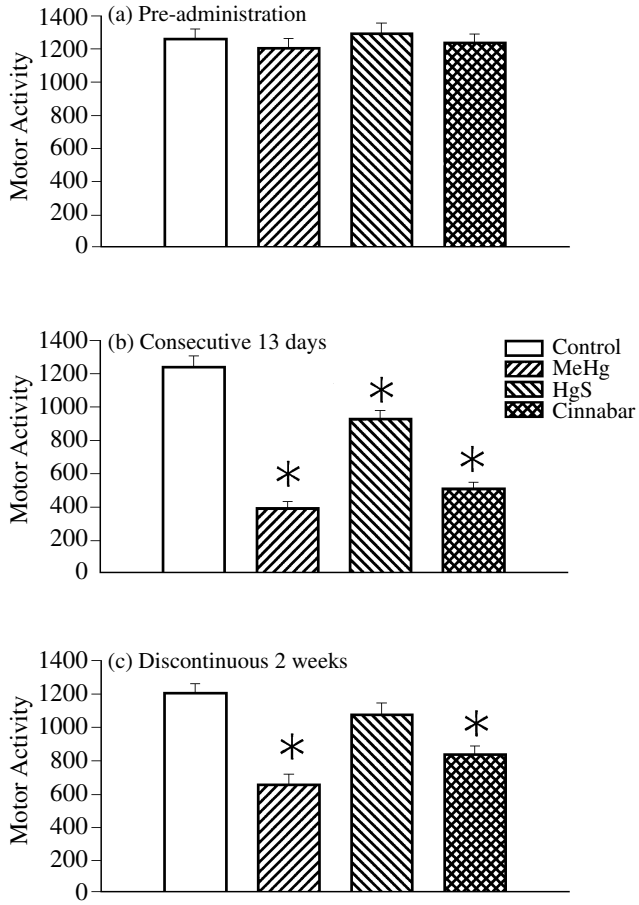

Fig. 5. Effects of mercurial compounds on spontaneous locomotion in rats. Ten rats per group were administered with either MeHg (2.0 mg/Kg), HgS (1.0 g/Kg) or cinnabar (1.0 g/Kg) or vehicle saline, respectively, for 13 consecutive days. Spontaneous total movement in an open-field arena performed by rats was recorded during a 60 min test session. The locomotion activity of each treated group was recorded before (a), 1 h after the last administration (b) and 2 weeks after discontinuous administration (c). Data are presented as mean ± SEM.
*$p < 0.05$ as compared with control.

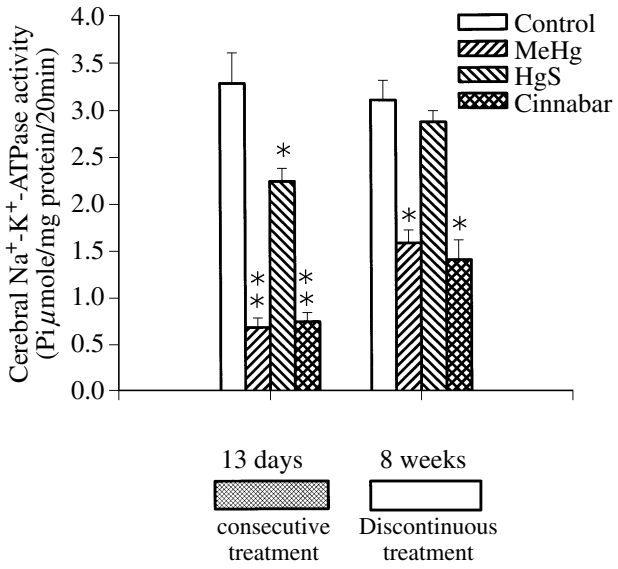

Fig. 6. Suppression of Na^+/K^+-ATPase activity in the cerebral cortex due to administration of mercurial compounds to rats. Effects of mercury compound administration on Na^+/K^+-ATPase activity in rats. Following MeHg (2.0 mg/Kg, IP), HgS (1.0 g/Kg, PO) and cinnabar (1.0 g/Kg, PO) administration for 13 consecutive days, the enzymatic activity was detected at various time courses. Data are presented as mean ± SEM.
*$p < 0.05$, **$p < 0.01$ as compared with the vehicle control.

MeHg ($F = 24.8$, 25.6 and 23.5, respectively, $p < 0.05$). HgS, however, reached 0.9 and 1.0 and 2.0 ppm, respectively ($F =$ 19.4, 18.4 and 18.7, respectively, $p < 0.05$), as compared to the control group (mercury content: 0.02, 0.025 and 0.1 ppm). On the other hand , we further found that when the mercury accumulated to 1.2 (not below 0.5) and 0.9 (not below 0.3) ppm in whole blood and the cerebral cortex, respectively, the rats showed serious neurotoxicity, which correlated with dysfunction of the active avoidance response as well as motor activity. Figure 7(b) shows that mercury accumulated significantly in these organs after 14 days of discontinuous administration.

IV. Discussion

In this study, we were the first to demonstrate that a high dose of cinnabar (1.0 g/kg) or MeHg (2.0 mg/Kg) not only produced central neurotoxicity, but also decreased body weight after 13 consecutive days. We monitored neurotoxicity based on the active avoidance response, locomotor activity and cerebral Na^+/K^+-ATPase activity. Since cinnabar, which is widely spiked in Chinese medicine, is a naturally occurring HgS containing other trace metals, purified HgS was also simultaneously tested in this study. The results obtained indicated that oral HgS (1.0 g/Kg) did not produce marked central neurotoxicity but apparently improved acquisition (during 13 consecutive days of administration) and long-term memory (2 – 33 weeks after cessation of treatment) as compared with the vehicle controls. The possibility that the analgesic effects of mercurial compounds contributed to prolonged latency for escape in active avoidance responses was considered. By means of tail flick thermal hyperalgesic tests, we demonstrated that by means of similar administration regimens, HgS and cinnabar had no analgesic effect, but that MeHg had a reversible analgesic effect, which restored after discontinuous administration. This finding indicated that the irreversible control neurotoxicity induced by MeHg was not totally due to the analgesic effect.

The main symptoms of mercury poisoning observed in persons exposed to it were headache, oral lesions, metalic taste, loss of memory, and irritability (Langauer-Lewowicka and Zajac-Nedza, 1997). This phenomenon seemed to be strictly related to the presence of mercury in the brain since it disap-

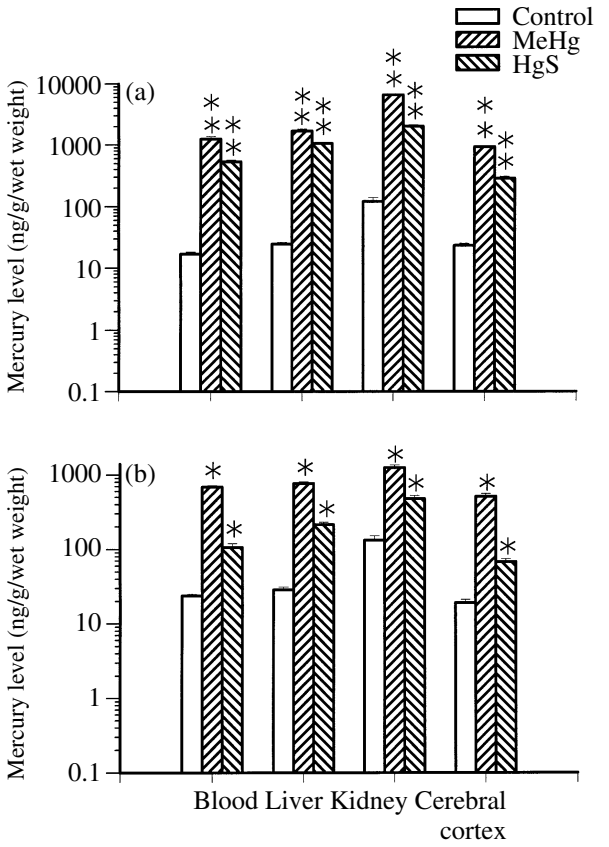

Fig.7. Mercury contents of various tissues in rats. Following daily oral dosing for 13 consecutive days with MeHg (2.0 mg/Kg, IP) and HgS (1.0 g/Kg, PO), mercury accumulation in the blood, liver, kidney and cerebral cortex of rats was measured 24 h (a) and 14 days (b) after the last administration using cold vapor AAS. Data are presented as mean ± SEM.

*$p < 0.05$, **$p < 0.01$ as compared with the vehicle control.

peared with normalization of mercury levels in the brain. In 20-trial active avoidance training, rats treated with two higher doses (cinnabar and MeHg) also showed impaired acquisition or suppressed retention performance when compared with the controls. Our data further confirmed that the effect of cinnabar or HgS was obviously different: both could induce long-term behavioural alterations that could be accurately displayed in locomotor activity and active avoidance performance. Eight weeks after cinnabar administration was discontinued, these rats still showed marked hypoactivity with significantly more disturbance of memory retention than did the control. The possible mechanisms of alteration in learning acquisition and long-term memory were assessed by measuring the combined effects of memory-enhancing agents upon altered pharmacological sensitivity in the neurotransmitter system.

Fipexide (10 mg/Kg) could abolish the amnestic effect of 6-hydroxydopamine (OHDA) and the memory-impairing effect of clonidine (Hansen, 1994), and restore to control values of the norepinephrine level in the frontal cortex and hippocampus (Stancheva *et al.*, 1993). In addition, it could also attenuate the impairment of acquired behaviour caused by sulpiride in pretrained rats (Marino *et al.*, 1990). The central cholinergic system has long been implicated in modulation of learning and memory processes in animals (Oda, 1999) and man (Pepeu and Spignoli, 1989). Cholinergic agents (physostigmine and arecoline) might ameliorate ethylcholine mustard aziridinium ion- or scopolamine-induced learning deficits (Nakamura *et al.*, 1992; Shannon *et al.*, 1999). These results, along with those of other previous reports on fipexide induced locomotor activity, allow us to conclude that the positive effect of this drug on learning acquisition is mediated, at least partially, by dopaminergic neurotransmission. Unfortunately, pretreatment with fipexide (10 mg/Kg) failed to cause an improvement in learning acquisition in cinnabar or MeHg treatment groups. Thus, the present study examined whether learning deficiency induced by mercuric compounds in rats could be majorly ameliorated by cholinergic system inactivation. Some reports also indicated that MeHg significantly reduced the maximum number of muscarinic receptors (Bmax) in the brain in 14 (53%) and 21-day-old rats (21%) in comparison with a saline-injected control (Ohta *et al.*, 1991). These results lead us to an hypothesis that cinnabar has value in treatments as a model of Chinese medicine for heavy metal-related acquisition for learning, and also suggest that memory deficits may be particularly susceptible to attenuation after non-cholinergic treatments. However, reduced cholinergic activity in the cerebral cortex may be, in part, responsible for the mercury-related decline in learning acquisition.

As reported previously, deltamethrin and aluminum exposure markedly decreased the Na^+/K^+-ATPase activity in the frontal cortex and hippocampus, associated with significant deficits in acquisition and retention of learned response in active avoidance situations (Husain *et al.*, 1996; Zatta *et al.*, 1995). Our results further indicated that mercuric compounds markedly impaired the learning decrease, which was accompanied by slight spontaneous locomotor activity, while HgS apparently improved the learning function. Although HgS reversibly and moderately inhibited cerebral Na^+/K^+-ATPase activity, this might have been tolerable inhibition. Whether HgS is responsible for potentiating memory-enhancing effects by producing reversibly, small changes in the locomotor activity and function of the cerebral Na^+/K^+-ATPase activity needs further investigation. Though the critical correlation between the biochemical parameters and learning ability was affected by cinnabar and MeHg, the results suggested that memory formation via learning was at least in part dependent on the Na^+/K^+-ATPase activity and played an important role in learning acquisition via the cholinergic system.

Elevation of the blood mercury level has been widely used as a biomarker for monitoring the extent of mercurial exposure (Grandjean *et al.*, 1999). In our experiment, we estimated the mercury level of blood and the cerebral cortex, which reached about 1.2 and 0.9 ppm, respectively, after 13 consecutive days of administration. Accordingly, central neu-

rotoxicity definitely appeared. Moreover, the correlation between the irreversible neurotoxicity and the subtained residue of mercury contents, ranging from 0.7 to 0.5 ppm in blood and the cerebral cortex, respectively, suggested that mercury accumulation played a major role in inducing central neurotoxicity, including electrical avoidance dysfunction.

In conclusion, it was clearly demonstrated that continuous oral administration of insouble HgS (1.0 g/Kg) for 13 days could increase the tissue level of mercury, which was well correlated with long-term memory retention. Concomitant study with low doses (2.0 mg/Kg) of MeHg showed that the well-absorbed MeHg led to a much higher tissue level of mercury, which accounted for the severe and irreversible neurotoxicity. Meanwhile, we believe that acetylcholine neurotransmission played a role in learning, memory and related cognitive processes while mercuric compounds impaired memory formation. Based on these results, it is tentatively concluded that the insoluble form of inorganic HgS can be absorbed from the gastrointestinal tract (G-I), and that its neurotoxic potency is about one thousandth or less that of soluble organic MeHg. These findings provide a basis for improved understanding of intoxication cases caused by the abuse of cinnabar (a naturally occurring HgS) prescribed in traditional Chinese medicine (Bellander *et al*., 1998).

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References

Annau, Z. and Cuomo, V. (1988) Mechanisms of neurotoxicity and their relationship to behavioral changes. *Toxicology*, **49**:219-225.

Bellander, T., Merler, E., Ceccarelli, F. and Boffetta, P. (1998) Historical exposure to inorganic mercury at the smelter works of Abbadia San Salvatore. *Ann. Occup. Hyg*., **42**:81-90.

Bowie, C., Hill, A. and Murray, V. (1998) The effect of a lindane and mercury polluting incident on the health of a community: the Somerton Health Survey. *Public Health*, **112**:249-255.

Genkova-Papazova, M.G. and Lazarova-Bakarova, M.B. (1996) Piracetam and fipexide prevent PTZ-kindling-provoked amnesia in rats. *Eur. Neuropsychopharmacol*., **6**:285-290.

Gerbi, A. and Maixent, J.M. (1999) Fatty acid-induced modulation of ouabain responsiveness of rat Na, K-ATPase isoforms. *J. Mem. Bio*., **168**: 19-27.

Gibbs, M.E. and Ng, K.T. (1979) Similar effects of a monoamine oxidase inhibitor and a sympathomimetic amine on memory formation. *Pharmacol. Biochem. Behav*., **11**:335-339.

Gilbert, S.G. and Grant-Webster, K.S. (1995) Neurobehavioral effects of developmental methylmercury exposure. *Environ. Health Persp*., **103**: 135-142.

Grandjean, P., Budtz-Jorgensen, E., White, R.F., Jorgensen, P.J., Weihe, P., Debes, F. and Keiding, N. (1999) Methylmercury exposure biomarkers as indicators of neurotoxicity in children aged 7 years. *Am. J. Epidem*., **150**:301-305.

Hansen, S. (1994) Maternal behavior of female rats with 6-OHDA lesions in the ventral striatum: characterization of the pup retrieval deficit. *Physiol. Behav*., **55**:615-620.

Hogg, S., Moser, P.C. and Sanger, D.J. (1998) Mild traumatic lesion of the right parietal cortex of the rat: selective behavioural deficits in the absence of neurological impairment. *Behav. Brain. Res*., **93**:143-155.

Hua, M.S., Huang, C.C. and Yang, Y.J. (1996) Chronic elemental mercury intoxication: neuropsychological follow-up case study. *Brain. Injury*., **10**:377-384.

Husain, R., Adhami, V.M. and Seth, P.K. (1996) Behavioral, neurochemical, and neuromorphological effects of deltamethrin in adult rats. *J. Toxicol. Env. Health*, **48**:515-526.

Iverson, F., Downie, R.H., Trenholm, H.L. and Paul, C. (1974) Accumulation and tissue distribution of mercury in the guinea pig during subacute administration of methyl mercury. *Toxicol. Appl. Pharm*., **27**:60-69.

Kang, Y.E. and Oransky, S.H. (1992) Chinese patent medicine as a potential source of mercury poisoning. *Vet. Hum. Toxicol*., **34**:235-238.

Lal, B., Gupta, A., Murthy, R.C., Ali, M.M. and Chandra, S.V. (1993) Aluminum ingestion alters behaviour and some neurochemicals in rats. *Indian. J. Exp. Biol*., **31**:30-35.

Langauer-Lewowicka, H. and Zajac-Nedza, M. (1997) Changes in the nervous system due to occupational metallic mercury poisoning. *Neurologia. Neurochirurgia. Polska*., **31**:905-913.

Lehotzky, K., Szeberenyi, J.M., Ungvary, G. and Kiss, A. (1988) Behavioral effects of prenatal methoxy-ethyl-mercury chloride exposure in rat pups. *Neurotoxicol. Teratol*., **10**:471-474.

Levesque, P.C. Hare, M.F. and Atchison, W.D. (1992) Inhibition of mitochondrial Ca^{2+} release diminishes the effectiveness of methyl mercury to release acetyl-choline from synaptosomes. *Toxicol. Appl. Pharm*., **115**: 11-20.

Liu, S.H., Lai, J.L., Lin, R.H., Lin, M.J. and Lin-Shiau, S.Y. (1997) Involvement of nitric oxide in the *in vivo* effects of lipopolysaccharide on the contractile and electrical properties of mouse diaphragm. *N-S. Arch. Pharmacol*., **356**:500-504.

Marino, A., Florio, T., Meucci, O., Landolfi, E., Grimaldi, M., Ventra, C. and Schettini, G. (1990) Fipexide improvement of cognitive functions in rat: behavioural and neurochemical studies. *Pharmacol. Res*., **22**:179-187.

Nakamura, S., Tani, Y., Maezono, Y., Ishihara, T. and Ohno, T. (1992) Learning deficits after unilateral AF64A lesions in the rat basal forebrain: role of cholinergic and noncholinergic systems. *Pharmacol. Biochem. Behav*., **42**:119-130.

Ndamba, J., Gomo, E., Nyazema, N., Makaza, N. and Kaondera, KC. (1997) Schistosomiasis infection in relation to the ABO blood groups among school children in Zimbabwe. *Acta. Tropica*. **65**:181-190.

Oda, Y. (1999) Choline acetyltransferase: the structure, distribution and pathologic changes in the central nervous system. *Pathol. Int*., **49**:921-937.

Ohta, H., Ni, X.H., Matsumoto, K. and Watanabe, H. (1991) Working memory deficit in aged rats in delayed nonmatching to position task and effect of physostigmine on performance of young and aged rats. *Jpn. J. Pharmacol*., **56**:303-309.

Page, S.J. and Terry, P. (1997) Conditioned grooming induced by the dopamine D1-like receptor agonist SKF 38393 in rats. *Pharmacol. Biochem. Behav*., **57**:829-833.

Pedersen, M.B., Hansen, J.C., Mulvad, G., Pedersen, H.S., Gregersen, M. and Danscher, G. (1999) Mercury accumulations in brains from populations exposed to high and low dietary levels of methyl mercury. Concentration, chemical form and distribution of mercury in brain samples from autopsies. *Int. J. Circ. Health*, **58**:96-107.

Pepeu, G. and Spignoli, G. (1989) Nootropic drugs and brain cholinergic mechanisms. *Prog. Neuropsychopharmacol. Biol. Psychiat*., **13**:S77-88.

Prakash, R., Singh, J. and Ratan, R. (1995) Ras-sindur and its standardization in Ayurvedic system of medicines. *Indian. Drugs*., **32**:225-226.

Raffaele, K.C., Asthana, S., Berardi, A., Haxby, J.V., Morris, P.P., Schapiro, M.B. and Soncrant, T.T. (1996) Differential response to the cholinergic agonist arecoline among different cognitive modalities in Alzheimer's

disease. *Neuropsychopharmacology*, **15**:163-170.

Rohn, T.T., Hinds, T.R. and Vincenzi, F.F. (1993) Ion transport ATPases as targets for free radical damage. Protection by an aminosteroid of the Ca^{2+} pump ATPase and Na^{+}/K^{+}-pump ATPase of human red blood cell membranes. *Biochem. Pharmacol*., **46**:525-534.

Santucci, B., Cannistraci, C., Cristaudo, A., Camera, E. and Picardo, M. (1998) Thimerosal positivities: the role of organomercury alkyl compounds. *Cont. Dermat*., **38**:325-328.

Shannon, H.E., Hart, J.C., Bymaster, F.P., Calligaro, D.O., DeLapp, N.W., Mitch, C.H., Ward, J.S., Fink-Jensen, A., Sauerberg, P., Jeppesen, L., Sheardown, M.J. and Swedberg, M.D. (1999) Muscarinic receptor agonists, like dopamine receptor antagonist antipsychotics, inhibit conditioned avoidance response in rats. *J. Pharmacol. Exp. Ther*., **290**:901-907.

Stancheva, S., Papazova, M., Alova, L. and Lazarova-Bakarova, M. (1993) Impairment of learning and memory in shuttle box-trained rats neonatally injected with 6-hydroxydopamine. Effects of nootropic drugs. *Acta. Physiologica. Pharmacologica. Bulgarica*., **19**:77-82.

Zatta, P., Zambenedetti, P., Pizziuti, A. and Perazzolo, M. (1995) Different effects of aluminum upon carbonic anhydrases and Na^{+}/K^{+}-ATPase activities in rat. *Neuroscience Lett*., **197**:65-68.

Zetterstrom, R. (1999) Child health and environmental pollution in the Aral Sea region in Kazakhstan. *Acta. Paediatrica.*, **88**(Suppl.):49-54.