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Effects of combined administration of thiol compounds and methylmercury chloride on mercury distribution in rats

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It was reported previously that the brain uptake of methylmercury was accelerated by combined administration of L-cysteine and depressed by the neutral amino acid, phenylalanine, but not by basic and acidic amino acids [1]. From these results, one can speculate that the blood–brain barrier transport system of neutral amino acid participates in some way in methylmercury penetration through the blood–brain barrier. Cysteine seems to be an important factor in the methylmercury uptake in the brain. On the other hand, it has been suggested that a methylmercury–glutathione complex is of importance for the methylmercury uptake in the kidney [2]. From studies on mercury accumulation at short times after the administration of inorganic mercury, Thomas and O'Tuama [3] suggested that uptake processes differ in different tissues. In order to clarify the methylmercury uptake process in the brain in comparison with other tissues, the effect of combined administration of L-, D-cysteine, glutathione or *N*-acetyl-L-cysteine and methylmercuric chloride on mercury distribution was investigated in rats.

Materials and methods

Methylmercuric chloride (purity: 98%), L-cysteine HCl, glutathione and *N*-acetyl-L-cysteine were purchased from Wako Pure Chemical Industries. D-Cysteine HCl was purchased from Nakarai Chemical Company. Each reagent was dissolved in phosphate buffered saline (pH 7.4). Kud: Wistar male rats (age 7 weeks) were used in this study. Either methylmercuric chloride (5 μmole/kg) alone, or methylmercury premixed with L-cysteine, D-cysteine, *N*-acetyl-L-cysteine or glutathione at the dose of 10 μmole/kg was injected i.v. into the rats. After 5 min, 3 and 24 hr, blood was collected from the abdominal vein of the rats under pentobarbital anesthesia. Then, the rats were perfused with saline, and the organs were removed for mercury determination. An aliquot of blood was centrifuged at 3000 rpm for 10 min for separation of plasma. Total mercury contents in the brain, kidney, liver, whole blood and plasma were determined by an oxygen combustion flameless atomic absorption system using Sugiyamagen Mercury analyzer MV250 R.

Statistical significance of mean values was calculated by Student's *t* tests and P values less than 0.05 were considered to be significant.

Results and discussion

All thiol compounds used in this experiment caused increased mercury contents in the brain, kidney and liver (Fig. 1), and lower mercury levels in the blood at 5 min (Fig. 2). The combined i.v. administration of methylmercury and thiol compounds significantly decreased whole blood: plasma ratio of mercury at 5 min (control, 169; L-cysteine, 77; D-cysteine, 92; *N*-acetyl-L-cysteine, 67; glutathione, 110). It has been reported that in the presence of glutathione or cysteine, the methylmercury uptake in red blood cells is depressed [3, 4].

The L-cysteine caused an additional increase in the mercury contents in the brain, while D-cysteine treatment showed the same mercury levels as the other thiol compounds. Such results mean that there was a stereospecificity of the blood–brain permeability to the methylmercury cysteine complex. Since it is known that barrier transport of amino acids is stereospecific [5], these results support our previous findings [1] that the blood–brain barrier transport system of amino acids might be involved in the methylmercury uptake in the brain. It has been reported that mercury–amino acid complexes might be involved in the transport of mercury into kidney cells via the same mechanism as amino acid themselves are transported [6]. In the present work, however, no stereospecific difference was observed in the renal uptake of methylmercury–cysteine complexes. The liver, blood and plasma contents also were not significantly different after simultaneous treatment with methylmercury and either D-, or L-cysteine. Alexander and Aaseth [2] reported that methylmercury glutathione complex in blood is rapidly extracted by the kidney. Our results showed that *N*-acetyl-L-cysteine and glutathione caused significantly higher mercury levels in the kidney at 5 min as compared with L- or D-cysteine treatment. The accumulation of methylmercury in the kidney may be a more complicated process and coupled with the metabolism of glutathione and cysteine in the kidney. Treatment with all of the thiol compounds increased the methylmercury uptake by the liver approximately equally. Alexander and Aaseth [2] reported that the mercury content in the liver was depressed at 1 hr in rats given methylmercury mercaptides. They suggested that liver cells are unable to take up glutathione and can export large amounts of the methylmercury glutathione complex rapidly into the

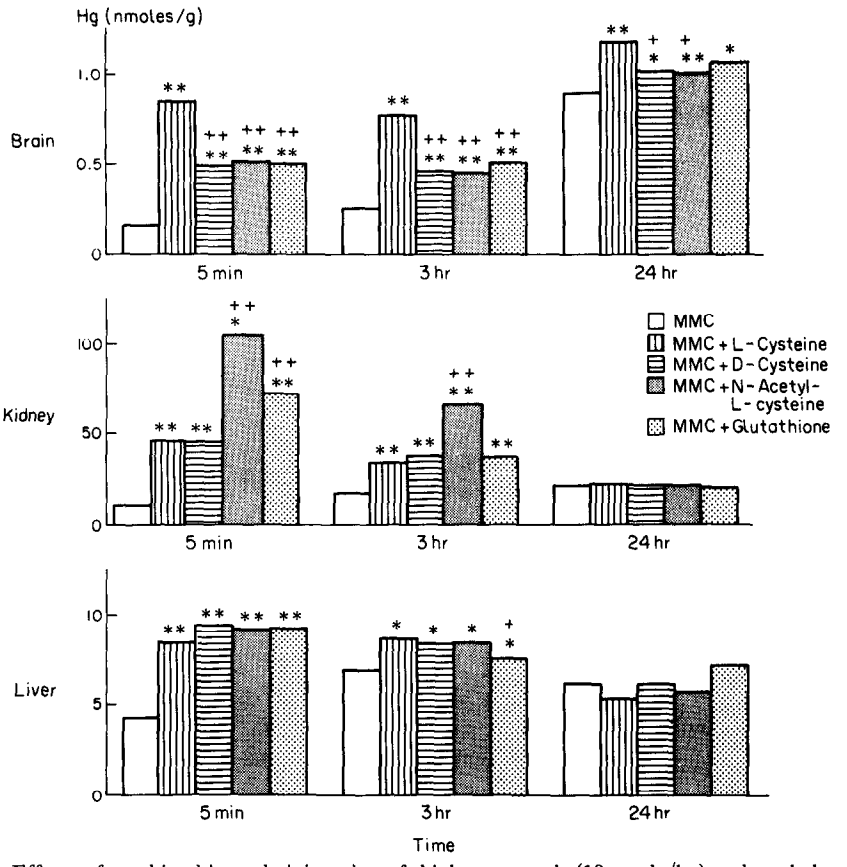

Fig. 1. Effects of combined i.v. administration of thiol compounds (10 μmole/kg) and methylmercuric chloride (5 μmole/kg) on the tissue mercury concentrations in the rats 5 min, 3 and 24 hr. Each value represents the mean obtained from 5 rats for 5 min and 3 rats for 3 and 24 hr: *$P < 0.05$; **$P < 0.01$: significant difference from rats administered methylmercuric chloride alone; $^{+}P < 0.05$; $^{++}P < 0.01$: significant difference from rats administered methylmercuric chloride and L-cysteine together.

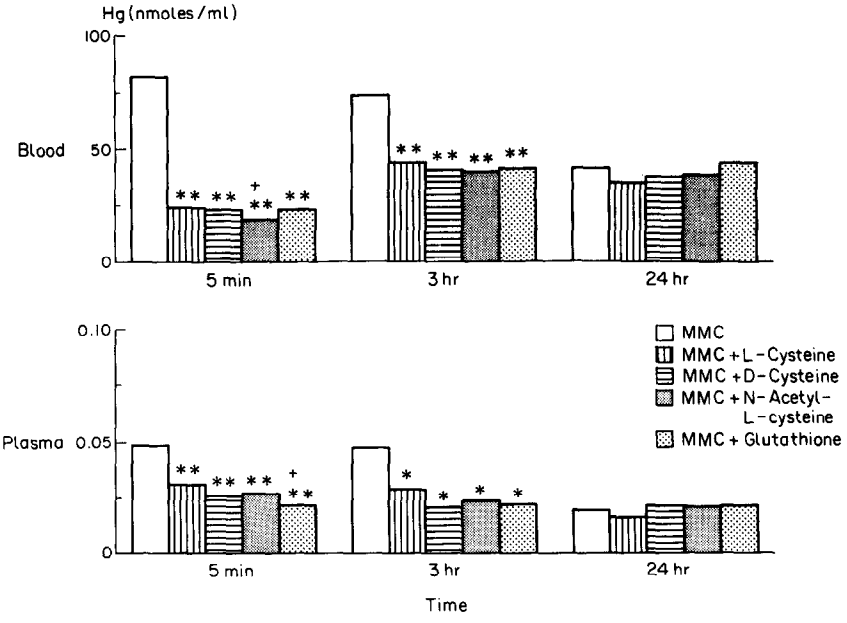

Fig. 2. Effects of combined i.v. administration of thiol compounds (10 μmoles/kg) and methylmercuric chloride (5 μmoles/kg) on blood and plasma mercury concentrations in the rats after 5 min, 3 and 24 hr. Each value represents the mean obtained from 5 rats for 5 min and 3 rats for 3 and 24 hr. *$P < 0.05$; **$P < 0.01$: significant difference from rats administered methylmercuric chloride alone; $^{+}P < 0.05$: significant difference from rat administered methylmercuric chloride and L-cysteine together.

bile, thus reducing the liver content of mercury. The reason for this discrepancy between the results of the present experiments with male rats and those of Alexander and Aaseth [2] with female rats is not clear, but could be due to the sex differences in mercury distribution in this species [7]. The differences in the time of the measurements seems unlikely to be a significant factor, since the increase in the hepatic concentration of mercury at 5 min after treatment with thiol compounds, as reported herein, also was observed at 3 hr (Fig. 1).

At 24 hr, the higher mercury level was still evident in the brain of the rats treated with thiol compounds, whereas the mercury levels of the other organs, plasma and blood did not show any significant difference at this time (Figs. 1 and 2). Magos *et al.* [8] reported that 30 min after cysteine injection the tissue cysteine level increased, and 2 hr after injection it declined nearly to the control level. When the cysteine level recovers to the control level, methylmercury may redistribute according to endogenous thiol levels. As shown in the present results, the effect of the exogenous thiol compounds on the liver, kidney and blood was temporary, but the effect on the brain lasted for up to 24 hr. This also suggests that the brain methylmercury uptake and elimination system is different from those of the other tissues.

In summary, the nature of the methylmercury uptake process in the brain was studied in comparison with that in the other tissues. Stereospecificity was found in the brain methylmercury uptake in the presence of methylmercury cysteine complex. This result may corroborate the assumption that the blood–brain barrier transport system participates in some way in the penetration of methylmercury to the brain.

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Effects of the hyperglycaemic agent 3-aminopicolinate on amino acid release by rat muscle *in vitro*

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3-Aminopicolinate is a hyperglycaemic agent *in vivo* that acts, in part, by enhancing gluconeogenesis [1, 2]. Studies to date have not ruled out an additional locus of action in peripheral tissues, and recently it has been suggested that an increase in the release of gluconeogenic precursors from peripheral tissues is the predominant action of the agent [3]. The release of glucogenic amino acids, and alanine in particular, from skeletal muscle is a significant determinant of whole body gluconeogenesis [4, 5]. The involvement of phosphoenolpyruvate carboxykinase in the proposed pathway for the synthesis *de novo* of muscle alanine [6–8] is noteworthy in view of the reported activation of the purified enzyme from rat liver by 3-aminopicolinate [9]. Indeed the hypoglycaemic analogue 3-mercaptopicolinate, an inhibitor of muscle phosphoenolpyruvate carboxykinase [8], diminishes muscle alanine release *in vitro* [6, 8] as well as decreasing hepatic gluconeogenesis through inhibition of the liver enzyme [10]. The effects of 3-aminopicolinate on muscle alanine (and glutamine) release using preparations of rat soleus and extensor digitorum longus (EDL) muscles *in vitro* were therefore investigated.

Materials and methods

Animals. Male Wistar rats, bred in the University of Surrey Animal Unit, were used at a body weight of 70–100 g. They were allowed water *ad libitum* during starvation for 48 hr and were killed at 10.00hr. Soleus and EDL muscles were dissected and incubated for 2 hr, after a 30 min preincubation period, as previously described [11]. Muscles from the contralateral limb of the same animal served as the control in incubations in the absence of 3-aminopicolinate.

Analytical methods. Alanine [6], glutamine [12] and tyrosine [13] were measured in $HClO_4$ extracts of the tissues and incubation media at the end of the incubation period. Phosphoenolpyruvate carboxykinase [14], pyruvate kinase and lactate dehydrogenase [15] were assayed in liver and muscle as described.

Chemicals. Sodium [^{14}C]bicarbonate was obtained from Amersham International (Amersham, Bucks., U.K.). Substrates, enzymes and coenzymes were from Boehringer Co. (London) Ltd. (Lewes, Sussex, U.K.) or Sigma Chemical Co. (Poole, Dorset, U.K.). 3-Aminopicolinic acid (m.p. 209°) was a generous gift from Dr. C. H. Reynolds (The Wellcome Foundation, Dartford, Kent, U.K.) and was prepared fresh and neutralized with NaOH immediately prior to its use in muscle incubations.

Results and discussion

Branched-chain amino acids are the most effective precursors for muscle alanine formation [4, 5]. 3 mM valine stimulated alanine release in incubations of soleus or EDL muscles to similar extents (Table 1). It has been proposed [6–9] that in starved rats valine can serve as a carbon source for alanine formation through its metabolism to citric acid cycle intermediates, and thence to pyruvate via oxaloacetate by the consecutive actions of phosphoenolpyruvate carboxykinase and pyruvate kinase. Transamination of pyruvate with glutamate (formed by transamination of valine) generates alanine via alanine aminotransferase.