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THE STIMULATION AND INHIBITION OF THE EXHALATION OF VOLATILE SELENIUM

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Abstract—Administration of methylmercury (1.5–24 μmol kg^{-1}; s.c.) to female rats simultaneously with Na_2 $^{75}SO_3$ (0.25 or 24 μmol kg^{-1}; s.c.) causes a dose-dependent increase in the exhalation of dimethylselenide. At the low selenite dose level, exhalation of ^{75}Se over a 24 hr period is about fourfold greater after treatment with 24 μmol kg^{-1} methylmercury than that (approximately 0.75% of the dose) in the controls, but excretion by other routes (urine, faeces) and the liver and kidney contents of ^{75}Se are not affected significantly. At the higher selenite dose level (24 μmol kg^{-1}) exhalation of ^{75}Se is correlated with the log dose of methylmercury. The faecal and urinary excretion remains essentially unaffected, and in rats treated with 24 μmol kg^{-1} methylmercury the ^{75}Se contents of the liver, kidneys and blood are reduced by 78%, 86% and 18% respectively. The effects of the alkylmercurial are not specific since, at this selenite dose level, ethylmercury increases the exhalation and decreases the liver and kidney contents of ^{75}Se approximately to the same extent as an equimolar dose of methylmercury. In methylmercury-treated and control animals dosed with 24 μmol kg^{-1} Na $^{75}SeO_3$ the exhalation of ^{75}Se is inhibited to the same extent by periodate-oxidized adenosine (PAD; 15 μmol kg^{-1}, i.p.) in the first 6 hr. Later inhibition is less pronounced in methylmercury-treated rats. Under these conditions PAD has little effect on the renal content, but increases the hepatic content of ^{75}Se. It seems, therefore, that the methylation of selenite occurs mainly in the liver and in both control and methylmercury-treated animals, *S*-adenosylmethionine is the major methyl donor. It is possible that methylmercury does not affect directly the methylation enzyme system but, by competition for protein sulphydryl groups, increases the availability of the intermediary selenide anion.

At the biochemical level the interaction between selenite and methylmercury includes alterations in their organ distributions [1–3], altered subcellular distribution of selenium [3, 4], the formation of an additive product, bismethylmercury selenide [5] and the stimulation of dimethylselenide exhalation [6]. In the work presented here three aspects of the last of these interactions have been investigated.

It has been reported that the exhalation of selenium was reduced fourfold when the dose of selenite was reduced from 28 μmol kg^{-1} to 11 μmol kg^{-1} [7]. As in previous work [6] the effect of methylmercury (24 μmol kg^{-1}) on selenium exhalation was studied only at one selenite dose level (24 μmol kg^{-1}) the possibility could not be excluded that methylmercury does not stimulate the methylation of selenium at low selenite concentration. In the present experiments the effects of different doses of methylmercury were compared in rats treated either with 24 μmol or 0.25 μmol kg^{-1} selenite.

A second problem was the role of methyl radical in the formation of dimethylselenide. As selenite does not increase the inorganic to organic mercury ratio in tissues [2], methylmercury cannot be the methyl donor for the methylation of selenium. Nevertheless the stimulation of selenium exhalation may be specific. This was investigated by comparing the effect of methyl- and ethylmercury on the exhalation of selenium.

The third problem involved the mechanism of methylation. To investigate this, studies were made on the effect of periodate-oxidized-adenosine (PAD) on the production of dimethylselenide in the presence and absence of methylmercury. PAD which, through inhibition of *S*-adenosylhomocysteine hydrolase, leads to the accumulation of *S*-adenosylhomocysteine and then to the inhibition of *S*-adenosylmethionine transmethylation reactions [8], has been shown to block the methylation of arsenic *in vivo* [9].

MATERIALS AND METHODS

All experiments were done with female rats (180–210 g body weight) of the laboratory (inbred) Wistar–Porton strain. MeHgCl (Pierce and Warriner, U.K.) and Na_2SeO_3 (BDH) were administered subcutaneously at the same time but at different sites as freshly prepared solutions in 2.5 ml saline kg^{-1}. Control animals were dosed with the same volume of saline. When the effects of ethylmercury and methylmercury were compared, both EtHgCl (Pierce and Warriner, U.K.) and MeHgCl were injected in the same volume of glycerinformal (Fluka AG, Buchs). The injection solutions of Na_2SeO_3 were supplemented with ^{75}Se-labelled selenite (Amersham International plc, Amersham, Bucks.) to give approximately 0.5 μCi ml^{-1}.

Periodate-oxidized-adenosine (PAD) was synthesized as described by Hoffman [8] and dried *in vacuo*. A suspension of the solid (100 mg) in saline

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(27 ml) was stirred for 15–20 min at room temperature and then centrifuged. The concentration of PAD in the supernatant solution, was determined by measurement of the absorbance at 259 nm of a 0.1 ml aliquot, diluted to 15 ml with M/15 phosphate buffer, pH 7.0, and from the molar extinction coefficient of 15400 M^{-1} cm^{-1} [8], the concentration varied between 5.2 and 6.0 μmol ml^{-1}.

Rats were injected i.p. with 15 μmol kg^{-1} PAD in 5 ml kg^{-1} saline 15 min before selenite. This dose produced no toxic symptoms. It was chosen after preliminary experiments had established that rats, in contrast with mice, which can tolerate 100 μmol kg^{-1} PAD without ill-effect [8, 9], either died, or became moribund within 24 hr after the administration of 50 μmol kg^{-1} PAD.

Animals after treatment were kept in glass metabolic cages (Metabowls, Jencons Scientific Ltd., Leighton Buzzard, Beds.) with free access to water and food. Urine and faeces were collected separately. Air was pumped at a rate of 2.5 l/min through the closed cages and 4 translucent vinyl tubes (8 mm inner diameter) each containing 1 g granular charcoal (8–20 Mesh) to absorb exhaled selenium.

Rats were decapitated 24 hr after treatment. Blood was collected from the severed vessels into beakers, kidneys and liver were removed. Blood and organs were weighed. Absorbers and biological samples including urine and faeces were assayed for ^{75}Se in a well-shaped NaI crystal of 8 cm inner diameter and depth by gamma counting with an efficiency of 85%.

Statistical evaluation followed standard methods described by Snedecor and Cochran [10].

RESULTS

Figures 1 and 2 show the exhalation of ^{75}Se after treatment with 0.25 or 24 μmol kg^{-1} Na_2 $^{75}SeO_3$.

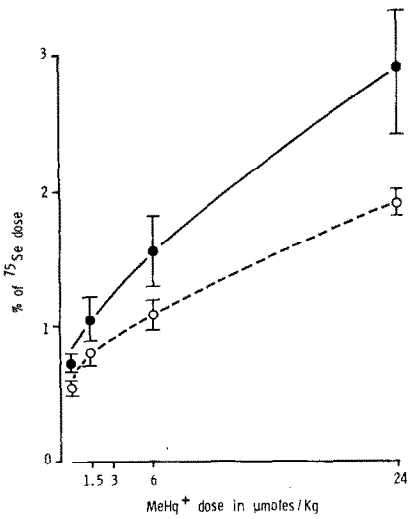

Fig. 1. The effect of methylmercury on the exhalation of ^{75}Se (0–6 hr, open circles; 0–24 hr, solid circles) after the s.c. administration of 0.25 μmoles/kg Na_2 $^{75}SeO_3$. Methylmercury was injected s.c. at the same time (but at different site) as selenite. SEMs are indicated by vertical bars.

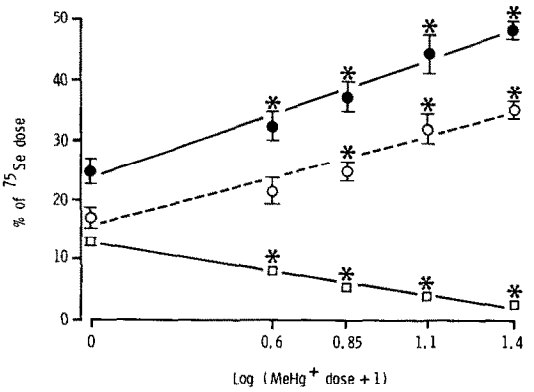

Fig. 2. The effect of methylmercury on exhalation of ^{75}Se (0–6 hr, open circles; 0–24 hr solid circles) after the s.c. administration of 24 μmoles/kg Na_2 $^{75}SeO_3$. Also shown is the liver content of ^{75}Se at 24 hr (open brackets). Methylmercury was injected s.c. at the same time (but different sites) as selenite. SEMs are indicated (when they are larger than the symbols) by vertical bars, and the significant difference ($P < 0.05$) (one-tailed test) from the group not receiving methylmercury by asterisks.

Irrespective of the dose of selenite, methylmercury increased the exhalation of ^{75}Se both in the 0–6 and in the 0–24 hr periods. After the smaller selenite dose, regression analysis gave intercepts of 0.67% and 0.88% of the dose for the 0–6 and 0–24 hr intervals respectively. Though these intercepts are higher than the control values, correlation between the dose of methylmercury and the exhalation of ^{75}Se was highly significant (one directional test: $P < 0.001$) with a correlation coefficient of $r = 0.879$ for 0–6 hr and $r = 0.849$ for 0–24 hr. Contrary to the small but significant effect on exhalation, methylmercury did not have a significant effect on the 24-hr urinary or faecal excretion and, at the end of this period, on the liver and kidney contents of ^{75}Se.

In rats treated with 24 μmol kg^{-1} Na_2 $^{75}SeO_3$ there was a close correlation between the exhalation of ^{75}Se and the log dose of MeHgCl. In order to make use of the control values exhalation (y) in Fig. 2 is plotted against $x = \log(\text{dose} + 1)$, that is the O dose was converted to 1 μmol kg^{-1}, 3 μmol kg^{-1} to 4.0 μmol kg^{-1} and so on. The regression line for 0–6 hr exhalation followed the equation $y = 15.2 + 13.74x$ with $r = 0.867$ and for the full 24 hr period $y = 23.6 + 17.6x$ with $r = 0.884$. There were inverse correlations between the dose of MeHgCl and the contents of ^{75}Se in liver ($r = -0.902$), the kidney ($r = -0.945$) and the blood ($r = -0.560$) contents of ^{75}Se. The regression line for liver ^{75}Se content is shown in Fig. 2. Control values for liver, kidney, blood, urinary and faecal ^{75}Se contents are compared with values obtained in rats treated with 24 μmol kg^{-1} MeHgCl in Table 1. It can be seen that MeHgCl had no appreciable effect on the faecal and urinary excretion of selenium and of the three tissues the decrease in the ^{75}Se content either in liver (78%) or kidneys (86%) was about 4.5 times greater than that in the blood (18%). In blood the effect of methylmercury became significant only at the two highest methylmercury doses.

Table 1. The effect of an equimolar dose of simultaneously administered MeHgCl on the liver, kidney, blood, urinary and faecal contents of ^{75}Se 24 hr after the injection of 24 μmol kg^{-1} Na_2 $^{75}SeO_3$

MeHgCl	No. of rats	^{75}Se in % of dose (mean ± SEM) Liver	Kidneys	Blood	Urine*	Faeces*
–	8	13.1 ± 1.1	3.4 ± 0.14	8.7 ± 0.24†	28.1 ± 2.7	1.7 ± 0.40
+	8	2.8 ± 0.23†	0.5 ± 0.02†	7.1 ± 0.22†	21.6 ± 2.8	1.5 ± 0.40

* One sample was collected from two rats.
† Significantly different from control, $P < 0.001$.

The effects of ethylmercury and methylmercury on exhalation, urinary excretion and the organ contents of ^{75}Se are compared in Table 2. The results show that the two alkyl-mercurials increased the exhalation and decreased the liver and kidney contents of ^{75}Se approximately to the same extent.

PAD inhibited the exhalation of ^{75}Se both in the first 6 hr and in the 6–24 hr periods (Table 3). Though methylmercury doubled the exhalation of ^{75}Se, the degree of inhibition by PAD was remarkably similar in the two conditions. In the first 6 hr PAD decreased exhalation by 97.4% in the absence and 97.6% in the presence of methylmercury. The corresponding reductions in the next 18 hr were 82.0% and 69.3%. PAD also significantly increased the liver and decreased the blood content of ^{75}Se both in the absence and presence of methylmercury treatment, but it increased the kidney content only in methylmercury treated rats.

DISCUSSION

It is well-established (see refs. 11 and 12) that the metabolism of SeO_3^{2-} to Se^{2-} involves the non-enzymic interaction of SeO_3^{2-} and GSH with the formation of the selenotrisulphide (dithioselane) derivative, GSSeSG, which is then reduced by NADPH and glutathione-reductase to H_2Se. Some of the derived Se^{2-} is incorporated into protein; the remainder is methylated by an enzyme-catalysed reaction, in which *S*-adenosylmethionine functions as the methyl donor, and is excreted either by exhalation as $(CH_3)_2Se$ or in the urine as $(CH)_3Se^+$ [11]. The distribution of selenium between these two excretory pathways indicates that the increase in the exhaled proportion of administered Na_2SeO_3 caused by increasing the dose [7] or, as shown in the present work, by the co-administration of MeHgCl (see Table 1) is not the result of a shift from urinary to pulmonary excretion.

The independence of urinary excretion from exhalation is also shown in Table 3. The urinary excretion of selenium remained unaffected by treatment with PAD and/or MeHgCl, while pulmonary excretion showed very wide variation (see Table 3).

As methylmercury competes with selenide for sulphydryl groups and by this competition a higher proportion of the dose may become accessible for methylation, the stimulatory effect of methylmercury on the exhalation of ^{75}Se was not necessarily an indicator of a direct interaction with an enzyme. It is certain that the effect is not specific to a methyl radical attached covalently to mercury, since ethylmercury is equi-active on a molar dose basis.

There was a two order difference in the exhalation of ^{75}Se relative to dose between rats treated with 24.0 μmoles/kg or 0.25 μmoles/kg Na_2SeO_3. In both cases methylmercury was able to stimulate the respiratory excretion of ^{75}Se. The only difference in the stimulatory effect was that after the lower dose of Na_2SeO_3 when exhalation could be increased by several times, there was a linear relation between exhalation and the dose of MeHgCl, while after the high Na_2SeO_3 dose the relationship was log dose. When the respiratory excretion of ^{75}Se was decreased to a low level by PAD, the stimulating effect of MeHgCl remained apparent.

The results of the present *in vivo* experiments with PAD confirm the function of S-adenosylmethionine in the methylation of Se^{2-} established by the *in vitro* studies [11, 12]. They suggest also that S-adenosylmethionine, although known to be depleted in the livers of selenite-treated rats [13], remains the major methyl donor in the methylation reaction, when exhalation of $(CH_3)_2Se$ is increased by treatment with alkylmercuricals.

Results in Table 3 show that the exhalation of ^{75}Se increased in the order of PAD < PAD + MeHgCl control < MeHgCl. A reverse order was expected in liver and kidneys as it has been shown

Table 2. Comparison of the effects of methyl- and ethylmercury on the exhalation and distribution or ^{75}Se after the s.c. administration of 24 μmol/kg Na_2 $^{75}SeO_3$ and 6.0 μmol/kg alkylmercury

Alkyl Hg	No. of rats	% of ^{75}Se dose (mean ± SEM) Exhaled air* 0–6 hr	0–24 hr	24 hr urine*	Liver	Kidney	Blood
–†	8	16.9 ± 1.48	24.7 ± 2.15	28.1 ± 2.71	13.1 ± 1.1	3.4 ± 0.14	8.7 ± 0.24
Methyl	10	23.5 ± 0.91	35.6 ± 0.82	26.3 ± 2.34	5.9 ± 0.32	1.0 ± 0.07	10.2 ± 0.48
Ethyl	10	21.8 ± 1.96	34.7 ± 1.97	25.4 ± 1.54	5.8 ± 0.33	1.1 ± 0.09	9.7 ± 1.38

* One sample was collected from two rats.
† Control values were taken from the experiment shown in Fig. 2.

Table 3. The effects of PAD and MeHgCl on the exhalation, urinary excretion, hepatic, renal and blood contents of ^{75}Se after the s.c. injection of 12 μmol/kg $Na_2\ ^{75}SeO_3$.

		% of ^{75}Se dose					
		Exhaled air*					
PAD	MeHgCl	0–6 hr N = 4	6–24 hr N = 4	24 hr urine N = 4	Liver N = 8	Kidneys N = 8	Blood N = 8
−	−	10.9	5.4	24.1	19.2	4.2	7.2
+	−	0.28†	0.97†	23.2	35.3†	5.1†	4.9†
−	+	19.4	12.7‡	22.6	11.3‡	2.6‡	7.8
+	+	0.47†	3.9†‡	23.6	30.7†	5.3†	5.0†
SEM§		17.6%	19.3%	1.46	1.8	0.2	0.3

PAD (15.0 μmoles/kg, i.p.) was given 15 min before and MeHgCl (6 μmoles/kg, i.p.) simultaneously with selenite.

* Numbers are geometric means and SEM is given in % of the geometric mean.

† Significant effect of PAD with analysis of variance or Studentised range statistics ($P < 0.05$).

‡ Significant effect of MeHgCl with analysis of variance or Studentised range statistics ($P < 0.05$).

§ Standard error of means derived from analysis of variance.

that soluble or microsomal liver fractions and the soluble kidney fraction can methylate selenide [12]. However, only the hepatic and not the renal ^{75}Se content followed the expected reverse order and this seems to support the view of Nakamuro *et al.* [14] that the methylation of Se^{2-} to the respiratory metabolite $(CH_2)_3Se$ is mostly carried out in the liver.

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