NOTE

Acute Endocrine Effects of a Single Administration of Methylmercury Chloride (MMC) in Rats

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Abstract

The acute effects of methylmercury chloride (MMC) on the endocrine functions were investigated with doses too small to cause any typical neurological dysfunctions. The hormones included PRL, LH, TSH, ACTH, corticosterone (Bk), testosterone (TLI), total thyroxine (T4) and free thyroxine (free T4). The changes in serum hormone levels from 1 hour through 10 days after a single injection of MMC (12 mg/kg s.c.) (Exp. 1), and dose-response relationships between MMC doses (2 to 16 mg/kg s.c.) and the serum hormone levels at 25 hours after MMC injection (Exp. 2) were examined. The acute effects revealed, which were all reversible, are summarized as follows; 1) MMC might directly inhibit thyroxine synthesis; 2) MMC could affect only stimulatory the pituitary-adrenal axis and PRL synthesis/release, the primary action site for which may be the CNS; and 3) the effects of the pituitary-gonadal axis were inconsistent and, therefore, this axis seems to be relatively resistant to MMC. On the other hand, the responses of PRL and TSH to TRH loading, which were examined for both groups in Exp. 3, suggested that MMC could not affect the metabolizing activity for serum PRL and TSH. The hormone levels of the MMC group enhanced by TRH recovered very rapidly as in the control group.

Thus, these acute and reversible endocrine effects seem to indicate relatively earlier development of possible chronic and irreversible effects on the endocrine functions when exposed to methylmercury chronically, and these should be examined further.

Though neurological/behavioral effects of methylmercury have been examined thoroughly, its effects on the endocrine functions have not been investigated well in general. However, Burton and Meikle (1980) reported that, even with low levels of methylmercury chloride (MMC) which caused no observable neurological effects,

it could diminish stress tolerance and lower sexual activity, and this might be attributed to MMC induced endocrine dysfunctions. Thus, clarification of this kind of atypical toxicity of methylmercury may provide helpful information not only for the basic knowledge of methylmercury intoxication but also for evaluating the methylmercury effects among people who have been exposed to it without any typical symptoms.

In the present preliminary study, therefore, the author intended to see the acute effects of MMC on the pituitary-adrenal, -gonadal and -thyroidal functions as well as on the synthesis/release of PRL in rats given a smaller dose of MMC which cannot induce any typical neurological symptoms.

Materials and Methods

Male Wistar rats with 8 to 9 weeks of age were used for all the experiments. Food and water were given *ad libitum* and light was on from 0700 to 1900 hours.

(Exp. 1)

Groups of 5 to 6 rats were sacrificed before and 1 hour, 1, 3, 7 and 10 days after s.c. injection of 12 mg/kg of MMC dissolved in 1.0 ml of 3% acetone-saline or of 1.0 ml vehicle only.

(Exp. 2)

To examine the dose-response relationships, 5 groups of 5 or 6 rats were treated with vehicle or 2, 4, 8 and 16 mg/kg of MMC s.c., respectively, and were sacrificed 25 hours later.

(Exp. 3)

Five groups of 3 to 6 rats were pretreated with vehicle or 12 mg/kg of MMC s.c. 26 hours prior to i.p. injection of TRH (protirelin tartrate, Takeda Yakuhin Kogyo). They were sacrificed at 0, 15, 30, 70 and 120 min after TRH loading.

In all the experiments above, animals were sacrificed at 10 to 11 a.m. for all the groups to eliminate the possible influence of circadian variations in the hormones. Therefore, MMC for the 1 day group in Exp. 1, for example, was injected with MMC 25 hours before sacrifice on the next morning. The animals were decapitated to collect the trunk blood and the anterior pituitaries, which were quickly frozen on dry ice after removal and kept at -20°C until assay. Serum and tissue PRL, LH and TSH were measured with NIADDK radioimmunoassay kits. Rat PRL-I-5, rat LH-I-6, rat TSH-I-6 and synthetic hACTH (1-39) were iodinated with Na¹²⁵I by chloramine T or lactoperoxidase methods (Kabuto *et al.*, 1977).

The anterior pituitary kept frozen was homogenized in a homogenizer (Nihonseiki Ltd). The

homogenate was centrifuged at 9,000 rpm for 10 min with a microcentrifuge (KM 15200, Kubota Seisakusho), and the supernatant was refrozen on dry ice. The supernatant was thawed again and the supernatant fluid was used for pituitary hormone assays, according to Wakabayashi (1977).

Serum Bk and TLI were measured by RIA with the antisera for Bk raised by N. Ohsawa *et al.* and for TLI by I. Morimoto. As the latter antiserum raised against testosterone-3-carboxymethyloxime-BSA has about 100% crossreactivity with 5- α dihydrotestosterone (Morimoto *et al.*, 1980), testosterone measured in the present study is called testosterone-like-immunoreactivity (TLI). Tritiated Bk and testosterone were purchased from New England Nuclear. T4 and free T4 were measured with RIA kits by Eiken ICL and Amersham International PLC., respectively.

No observable neurological/behavioural dysfunctions such as tremor, paralysis or anorexia that have been reported as characteristic of methylmercury intoxication were found throughout the experiments.

Results are expressed as mean $\pm$ SE, and statistical analyses were performed by Student's *t*-test or Aspin-Welch's *t*-test. Duncan's multiple range test was also applied where the variance values are indicated by *, ** and ***, which mean $p < 0.05$, $p < 0.01$ and $p < 0.001$, respectively.

Results

Acute Endocrine Changes (Exp. 1)

As shown in Table 1, serum PRL and TSH levels were high from 1 to 7 days after the MMC treatment, but the increases were significant only at 7 days owing to large variations. LH increased slightly at 1 hour and 7 days. ACTH did not change significantly, though a slight increase was observed at 1 hour. On the other hand, as shown in Table 2, Bk showed two increases at 1 hour and 3 days. TLI decreased only at 1 day and T4 did so from 1 hour through 3 days with a trough at 1 day. Free T4 also decreased only at 1 day. All of these endocrine changes, however, were reversible.

Table 1. Serum Pituitary Hormone Levels after MMC Injection

Time after MMC	PRL (ng/ml)	LH (ng/ml)	TSH (ng/ml)	ACTH (ng/ml)
Control	2.35±0.45(5)	0.29±0.01(5)	0.97±0.21(5)	3.77±0.87(5)
1 hour	3.25±0.53(6)	0.55±0.04(6)***	1.02±0.22(6)	5.29±0.81(6)
25 hours	16.65±6.01(5)	0.42±0.06(5)	1.94±0.27(5)	4.33±0.94(5)
3 days	10.60±4.18(5)	0.39±0.05(5)	2.71±0.72(5)	3.59±0.62(5)
7 days	13.15±2.44(5)*	0.53±0.08(5)*	3.65±0.57(5)**	3.84±0.55(5)
10 days	5.20±1.50(5)	0.44±0.07(5)	2.09±0.33(5)	3.46±0.69(5)

Note: The values are expressed as the mean±SE (number of animals).

Table 2. Serum Bk, TLI and T4 and Free T4 Levels after MMC Injection

Time after MMC	Bk (μg/dl)	TLI (ng/ml)	T4 (μg/dl)	Free T4 (μg/dl)
Control	4.66±0.41(5)	2.14±0.21(5)	5.24±0.25(5)	2.32±0.52(5)
1 hour	14.25±1.56(6)***	5.49±1.65(6)	3.73±0.17(6)***	1.40±0.16(5)
25 hours	5.24±0.54(5)	1.54±0.09(5)*	1.65±0.17(5)***	0.63±0.15(5)*
3 days	7.27±0.21(5)***	2.71±0.36(5)	3.64±0.16(5)***	1.62±0.09(5)
7 days	4.68±0.29(5)	5.53±1.45(5)	4.50±0.38(5)	1.53±0.08(5)
10 days	4.40±0.06(5)	4.32±1.45(5)	5.67±0.34(5)	1.56±0.10(5)

Note: The values are expressed as the mean±SE (number of animals).

Table 3. Dose-Response Relationships between MMC dose and Serum Pituitary Hormone Levels

Dose of MMC	PRL (ng/ml)	LH (ng/ml)	TSH (ng/ml)
Control	2.60±0.28(5)	0.33±0.05(5)	1.61±0.30(5)
2 mg/kg	3.32±0.95(5)	0.92±0.08(5)	2.03±0.30(5)
4 mg/kg	3.82±1.45(5)	0.45±0.04(5)	2.85±0.30(5)
8 mg/kg	2.34±0.41(6)	0.50±0.07(6)	2.04±0.43(6)
16 mg/kg	6.15±0.59(6)***	0.55±0.11(6)	4.07±0.78(6)*

Note: The values are expressed as the mean±SE (number of animals).

Table 4. Dose-Response Relationships between MMC dose and Serum Bk, TLI and T4 Levels

Dose of MMC	Bk (μg/dl)	TLI (ng/ml)	T4 (μg/dl)
Control	8.30±1.93(5)	1.64±0.63(5)	4.12±0.47(5)
2 mg/kg	5.78±1.79(5)	1.68±0.48(5)	4.30±0.22(5)
4 mg/kg	6.69±1.13(5)	1.38±0.65(5)	3.61±0.38(5)
8 mg/kg	8.10±1.39(6)	2.89±0.78(6)	3.10±0.13(6)*
16 mg/kg	8.35±1.58(6)	1.17±0.42(6)	2.27±0.18(6)**

Note: The values are expressed as the mean±SE (number of animals).

Dose-Response Relationships (Exp. 2)

MMC, only at the highest dose (16 mg/kg), caused significant increases in PRL and TSH at 26 hours after the injection (Table

3). T4 was decreased at 8 and 16 mg/kg (Table 4). LH, TLI and Bk did not show any significant change at any dose. Accordingly, only the changes in TLI after

MMC in Exp. 1 were not reproduced in this experiment.

TRH Loading Test after MMC Pretreatment (Exp. 3)

Figs. 1 and 2, indicate the responses of serum TSH and PRL to TRH loading in rats with and without MMC pretreatment of 12 mg/kg s. c. 26 hours before sacrifice,

respectively. The slight enhancement of TSH release by MMC was found in the pre-TRH level of serum TSH in Fig. 1, the possibility of which was suggested in Exps. 1 and 2. TSH response in the MMC group was found to be markedly greater than that in the control group, while no significant difference was observed in PRL response to TRH in the two groups.

Fig. 1. Serum TSH responses to TRH loading ($10 \mu\text{g}/\text{rat}$) in rats with and without pretreatment with MMC ($12 \text{ mg}/\text{kg s. c.}$) 26 hours before sacrifice are shown. In controls, as shown by closed circles, mean serum TSH levels rose significantly at 15, 30 and 70 min when compared to the pre-TRH one, while in the MMC group TSH increased significantly only at 15 and 30 min, as shown by open circles. On the other hand, between the two groups at each time after TRH, the differences were significant at pre-TRH and 30 min.

Fig. 2. Serum PRL responses in the same TRH loading test as in Fig. 1 are indicated. In controls, mean PRL increased significantly at 15, 30 and 120 min, while no significant change was found in the MMC group. The pre-TRH PRL in the MMC group was significantly higher than that in the controls.

The anterior pituitary contents of TSH and PRL, as shown in Figs. 3 and 4, did not change significantly in response to TRH in the controls or in the MMC group. However, PRL content was less in the MMC group from pre-TRH through 70 min after TRH, which, though not significant, may have reflected the enhanced release of PRL by MMC before TRH loading, as shown in the pre-TRH level of serum PRL in Fig. 2.

In both cases of TSH and PRL response to TRH, the serum hormone levels were enhanced by TRH at 15 and 30 min. after TRH recovered rapidly to the pre-TRH levels in the MMC group as in the control group. This rapid recovery seems to indi-

cate that MMC did not apparently affect the metabolism of the two hormones.

Discussion

A single administration of 12 mg/kg of MMC was shown to enhance serum TSH from 25 hours through 10 days, though a significant increase was found only at 7 days. Serum TSH was increased significantly even at 25 hours with a higher dose (16 mg/kg). When the simultaneous changes in serum T4 and free T4 are compared with that of TSH as in Fig. 5, MMC seemed to affect the thyroid to minimize serum T4 levels, and the thyroid hormone depletion

Fig. 3. Responses of TSH content in the anterior pituitary in the TRH loading test as in Fig. 1 are shown. No significant changes in response after TRH in the controls or in the MMC group were observed. And no difference between the two groups was found at any time after TRH.

Fig. 4. Responses in PRL content in the anterior pituitary in the TRH loading test as in Fig. 1 are shown. Excluding the value at 120 min after TRH in the MMC group, no significant change nor difference was found.

Fig. 5. Simultaneous changes in serum TSH, T4 and free T4 which are indicated in Table 1 and 2 are shown.

around 25 hours might cause the TSH release which was observed from 1 to 7 days. This agreed well with the findings that methylmercury acetate has a high affinity with the thyroid and is concentrated especially in the thyroidal cells and not in its colloid fraction (Suzuki *et al.*, 1966) and also that ^{131}I uptakes by the thyroids in mice are reduced at 4 and 24 hours after 10 mg/kg of MMC, which may be attributed to a coupling defect in the synthesis of iodothyronines caused by MMC (Kawada *et al.*, 1980).

The decrease in serum TLI at 25 hours in Exp. 1 was not reproduced even with 16 mg/kg of MMC in Exp. 2. Since the simultaneous changes in serum LH in Exp. 1 seem also to be independent of those in TLI, MMC effects on the pituitary-gonadal axis failed to be clarified by the present study. However, LH content in the anterior pituitary in Exp. 2, though data are not shown in results, was found to be significantly enhanced with 16 mg/kg of MMC.

Therefore, MMC effects on this axis are to be investigated further.

There were two increases in serum Bk—a large one at 1 hour and a small but significant one at 3 days after MMC, while ACTH showed an insignificant tendency to increase except at 1 hour after MMC. However, it is known that peripherally administered MMC could be distributed into the CNS easily even at 60 min after MMC (Ukita, 1971). On the other hand, it is also known that MMC could not affect the metabolism of serum Bk, since MMC treatment in adrenalectomized rats did not change the half life of tritiated corticosterone administered into the blood (Burton and Meikle, 1980). Therefore, it might be possible that MMC acted primarily on the CNS to stimulate ACTH secretion at least at 1 hour after MMC, though the small increase in Bk at 3 days cannot be explained by the same mechanism.

On the other hand, the marked increase in mean serum PRL from 1 hour through

7 days, though a significant increase was found only at 7 days, may possibly have reflected the CNS action of MMC, since the synthesis/release of pituitary PRL in males is regulated exclusively by the CNS.

Thus, in the present study, the multiple endocrine effects of a single injection of MMC have been shown especially during the phase up to 10 days after MMC without any observable neurological/behavioural dysfunctions. These acute and reversible endocrine effects suggest relatively earlier development of possible chronic and irreversible effects on endocrine functions, which should be examined further simultaneously with those on the neurological/behavioural functions.

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