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DEPRESSION OF DRUG METABOLISM IN THE MOUSE BY A COMBINATION OF *Mycobacterium butyricum* AND ANAESTHETICS

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1 Subcutaneous injection of *Mycobacterium butyricum* suspended in mineral oil into the mouse hind paw caused an oedematous local inflammation. Hind paw swelling was maximum 5 days after injection and was still apparent at day 30.

2 Drug metabolism *in vivo* (as monitored by ketamine- or pentobarbitone-induced sleeping times) was not affected by the inflammatory disease. However, administration of ketamine or pentobarbitone at day 1 led to significantly elevated sleeping times when the mice showing local inflammation were retested at day 5 with the anaesthetics.

3 Indomethacin inhibited hind paw oedema in the mouse but did not affect ketamine-*Mycobacterium butyricum*-induced depression of drug metabolism.

4 Prolongation of ketamine-induced anaesthesia by combination with *Mycobacterium butyricum* at day 5 correlated with the degree of hind paw inflammation at this time.

5 The data suggest that anaesthetics (i.e., ketamine and pentobarbitone) may sensitize hepatic membranes to the effect of *Mycobacterium butyricum* or some toxic compound elaborated during the active phase of inflammation.

Introduction

In recent years inhibition of drug metabolism, i.e., the mixed function oxidase (MFO) system, in rats affected with experimentally-induced inflammatory diseases has generated considerable interest. Rats made polyarthritic by the injection of *Mycobacterium* in oil (Freund's adjuvant) have impaired hepatic (Morton & Chatfield, 1970; Whitehouse & Beck, 1973; DiPasquale, Rassaert, Welaj & Gingold, 1975; Cawthorne, Palmer & Green, 1976) as well as pulmonary MFO metabolizing capacity (Carlson & Ciaccio, 1975). Arthritis in rats induced by *Mycoplasma arthritidus* (Cawthorne *et al.*, 1976) or 6-sulphonanilamidoindazole (Swingle, Chang & Erikson, 1978) was associated with reduced metabolism of drugs. In addition to these chronic systemic diseases, transitory local inflammation may inhibit the drug metabolizing system of the rat (Beck & Whitehouse, 1974).

The biochemical effects of inflammation in the mouse have been poorly studied. Graeme, Fabry & Sigg (1966) found that the injection of Freund's adjuvant into the hind paw of this species resulted in a localized inflammatory reaction, which was inhibited by some anti-inflammatory compounds, but did not cause disseminated arthritis. To our knowledge an extension of these observations, relative to the effects on drug metabolism in the mouse, has not been made.

During our investigation of metabolic changes that occur in the mouse with *Mycobacterium*-induced inflammation, we observed an interesting interaction of this disease with ketamine (a nonbarbiturate general anaesthetic) and pentobarbitone (a barbiturate sedative-hypnotic). Both drugs act rapidly and are rapidly metabolized by the MFO metabolizing system.

Methods

Male CFW mice (Charles-River), weighing initially between 20 and 25 g, were randomly assigned to treatment groups and housed in an area of controlled light, temperature and humidity. Purina mouse chow and tap water were provided *ad libitum*.

Inflammation was produced by a single subcutaneous injection of 0.1 mg of *Mycobacterium butyricum* (*M. butyricum*, Difco Laboratories), suspended in mineral oil, into a foot pad of the right hind paw. Normal control animals were injected with an equal volume (20 µl) of mineral oil vehicle. Hind paw volumes were determined plethysmographically by a

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modified method of Van Arman, Begany, Miller & Pless (1965). A mercury well was connected to a pressure transducer (Statham Model P23AC), an output from the transducer was led to a polygraph (Grass Model 5D) equipped with a low-level d.c. preamplifier (Grass Model 5P1E). Throughout each experiment the instrument was calibrated repeatedly by the addition and withdrawal of known volumes of mercury (0 to 2 ml). For measurement of hind paw volumes, the right hind limb was immersed up to the hairline in the mercury pool. Determinations of paw volumes and body weights were made at regularly spaced intervals throughout each experiment.

Drug metabolism *in vivo* was measured by the duration of the sleeping time after administration of ketamine hydrochloride (150 mg/kg, i.p.) or sodium pentobarbitone (40 mg/kg, i.p.). These anaesthetics were prepared by dilution of ketamine hydrochloride injection (Ketalar Injection, 50 mg/ml, Parke-Davis) and sodium pentobarbitone injection (Nembutal Injection, 50 mg/ml, Abbott Laboratories) with 0.9% w/v NaCl solution (saline). The injection volume for all animals was 10 ml/kg.

Indomethacin (Merck, Sharp and Dohme) at doses of 1 and 3 mg/kg, intraperitoneally was used as a standard anti-inflammatory drug in an attempt to inhibit the inflammatory response and changes in drug metabolism.

Data were analyzed by analysis of variance (Snedecor & Cochran, 1967) after heteroscedastic variances were normalized by logarithmic transformation.

Results

Subcutaneous injection of *M. butyricum* suspended in mineral oil caused a significant oedematous reaction of the injected hind paw (22% increase in volume, $P < 0.001$) within 2 h. Maximum hind paw swelling generally occurred at 5 days after injection (Figure 1); the paw volumes approximately doubled, from 0.28 ± 0.01 ml (preinjection mean volume $\pm$ s.e. mean) to 0.53 ± 0.03 ml. The observed inflammation remained unchanged until after day 15. At day 30, inflammation persisted (0.44 ± 0.02 ml) but had subsided. No other external inflammatory lesions were observed. Body weight gain was unaffected throughout the 30 day observation period.

The effect of repeated doses of ketamine hydrochloride (150 mg/kg, i.p.) on normal control and mice showing signs of inflammation is shown in Table 1. There was no significant change in sleeping times of the control animals throughout this study; liver enzyme induction was not observed. At day 5, mice with local inflammation had a significantly elevated sleeping time ($P < 0.001$) suggesting depression of the hepatic drug metabolizing system; however, at day 9 and thereafter, the response to the ketamine challenge was not significantly different from the respective control group. Onset of the loss of righting reflex was the same in all experimental groups. In addition, the use of ketamine had no effect on the course or severity of the inflammatory reaction.

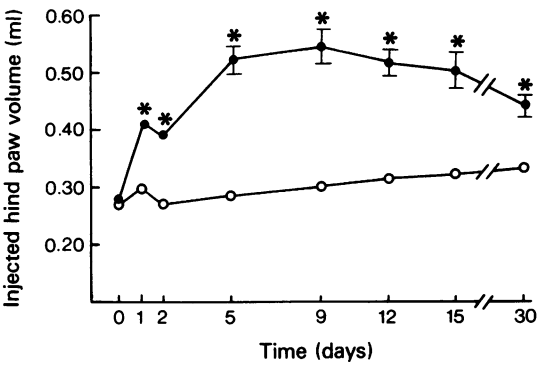

Figure 1 Hind paw volumes of mice after the injection of mineral oil (○) or *Mycobacterium butyricum*, 0.1 mg/mouse (●), at day 0. Each point represents the mean of 20 animals; vertical lines show s.e. mean. Significant difference from control: $*P < 0.001$.

From the results in Table 1 it appeared that the maximum depression of the MFO metabolism in *M. butyricum*-treated mice was at day 5. We conducted a preliminary experiment in which mice (oil-treated and *M. butyricum*-treated, 10 animals per group) were injected with ketamine only at day 5. The mean $\pm$ s.e. ketamine-induced sleeping times of control animals (21.9 ± 2.3 min) and mice with hind paw inflammation (22.8 ± 2.7 min) were not significantly different. Therefore, it would appear that prior exposure to ketamine (at day 1) was necessary in order to obtain elevated sleeping times at day 5.

Table 1 Ketamine sleeping time in mice* with *Mycobacterium butyricum*-induced inflammation

	Ketamine sleeping time (min $\pm$ s.e. mean)	
Day	*Control*	*Inflamed*
1	17.2 ± 0.7	19.5 ± 1.1
5	17.0 ± 0.9	31.1 ± 2.4‡
9	17.7 ± 1.3	21.8 ± 1.8
15	14.6 ± 1.7	16.3 ± 1.1
30	14.6 ± 1.9	17.6 ± 1.6

* Twenty mice were used in each group and repeatedly challenged with ketamine hydrochloride (150 mg/kg, i.p.) on the days indicated.
‡ Significantly different from control at $P < 0.001$.

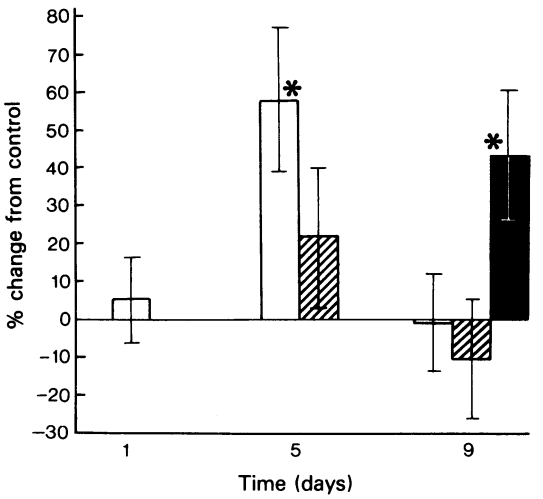

Figure 2 The effect of single and multiple doses of ketamine hydrochloride (150 mg/kg, i.p.) on ketamine-induced sleeping time in mice with hindpaw inflammation. All values are the mean from 10 mice in each group; vertical lines show s.e. means. The values are expressed as a percentage change from each respective control (oil-injected) group; open columns, ketamine administered on days 1, 5 and 9; hatched columns, ketamine administered on days 5 and 9; solid columns, ketamine administered on day 9 only. Significant difference from control: $*P < 0.05$.

Figure 2 shows data from a combination experiment which are largely compatible with this view. Ketamine administered only once, either on day 1 or 5, to mice with inflammation resulted in a sleeping time that was not significantly different from that of the corresponding oil-treated groups. Administration of the anaesthetic at day 5 (when the animals had also been exposed to the drug at day 1) resulted in prolonged sleeping times. Exposure to ketamine at day 5 or days 1 and 5 resulted in normal sleeping times when the mice were further challenged at day 9. Although a group of naive mice that were given ketamine 9 days after *M. butyricum* had sleeping times that were significantly different from their respective controls, in subsequent experiments these prolonged sleeping times were not observed at day 9.

By use of doses of *M. butyricum* from 0.05 to 0.4 mg/mouse, a dose-inflammatory response relationship was obtained. Prolongation of ketamine-*M. butyricum*-induced anaesthesia at day 5 correlated with the degree of hind paw inflammation (Figure 3).

Six day treatment (days −1 to 4) with indomethacin (1 mg/kg, i.p.) inhibited 5-day hind paw oedema by 13%; the 3 mg/kg dose produced a 26% inhibition. This potent anti-inflammatory agent did not affect ketamine-*M. butyricum*-induced prolongation of sleeping time.

It was of interest to compare the effects seen after ketamine with those of a barbiturate anaesthetic. Oil-treated and *M. butyricum*-treated mice were tested with sodium pentobarbitone (40 mg/kg, i.p.) either at days 1, 5 and 9, or 5 and 9, or 9 only (Table 2). Control mice receiving 3 doses of the drug showed hepatic enzyme induction as indicated by the successive reduction in mean sleeping times and the significantly shorter time at day 9. In the group of mice with inflammation, this effect did not occur: thus, a significant inhibition ($P < 0.05$) of the effect of pentobarbitone was observed in these mice at days 5 and 9. In the other two groups, the response to pentobarbitone was not significantly different in the mice with inflammation, as compared with corresponding controls. Therefore, as described for ketamine, exposure of mice with local inflammation to pentobarbitone (at day 1) was required to produce the subsequent inhibition of drug metabolism.

Discussion

Graeme *et al.*, (1966) found that the injection of *M. butyricum* into the hind paw of the mouse resulted in swelling of the limb which was maximal within 24 to 48 h after injection. No change was observed until day 14; measurements taken at day 33 indicated that the inflammation persisted, but had slowly waned. Our data are essentially in agreement with those of Graeme *et al.* (1966) except that the oedematous reaction to the *M. butyricum* injection was maximum in most cases at 5 days. In one experiment the oedema did not increase beyond the degree normally observed at day 2. The reasons for this occurrence are unknown, but this inflammatory pattern was similar to that observed by other investigators (Graeme *et al.*, 1966).

Depression of drug metabolism and disseminated polyarthritic disease are well-known phenomena associated with *M. butyricum* injection into rats. Neither of these effects was observed in the mouse. Depression of drug metabolism was observed 5 days after initiation of inflammation only after mice were previously exposed to ketamine (day 1). In one experiment prolonged ketamine sleeping times were observed at day 9 without the administration of ketamine at day 1; however, this effect was not reproduced in subsequent experiments. Data from pentobarbitone-treated mice are similar to results obtained with ketamine. Prior exposure to pentobarbitone (at day 1) in animals with hind paw inflammation was necessary in order to alter the metabolic profile which occurred in normal mice, i.e., enzyme induction. Interestingly, the pentobarbitone effect appears longer lasting than with keta-

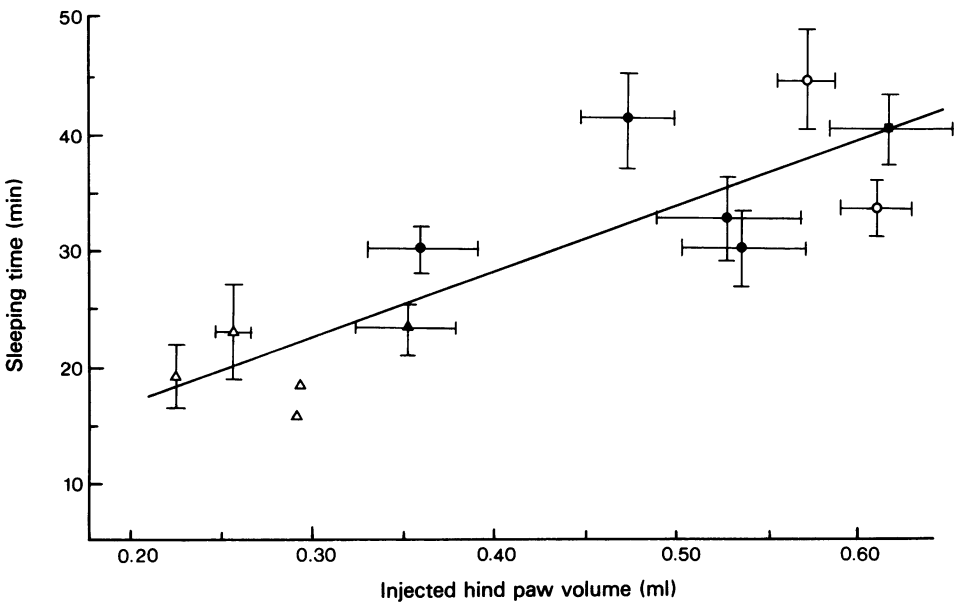

Figure 3 Relationship between ketamine-induced sleeping times in mice and hind paw inflammation 5 days after the injection of mineral oil (△) or *Mycobacterium butyricum*, 0.05 (▲), 0.1 (●), 0.2 (○), 0.4 (■) mg/mouse; all animals had been challenged with ketamine at day 1. Each point represents the mean of 10 animals; the horizontal and vertical lines represent s.e. means. Equation for the regression line: $y = 5.53 + 55.92x$; correlation coefficient (r), 0.851 (significantly different from 0 at $P < 0.01$).

mine, since sleeping times were significantly different in the inflamed group at day 9.

The severity of hind paw inflammation (day 5) from the different doses of *M. butyricum* correlated with the degree of depression of ketamine metabolism at day 5. This suggests a causal effect of inflammation on the metabolic alterations. Beck & Whitehouse (1974) observed a significant correlation between the severity of the injected rat hind paw inflammation and increase in hexobarbitone sleeping times in

Table 2 The effects of single and multiple doses of pentobarbitone on pentobarbitone sleeping time in mice with *Mycobacterium butyricum*-induced inflammation

Treatment and dose (Days drug used)		*Sleeping time (min ± s.e. mean) on* Day 1	Day 5	Day 9
Pentobarbitone, 40 mg/kg, i.p. (Days 1, 5 and 9)	Control	53.1 ± 6.4 (8)†	43.8 ± 4.0 (8)	25.3 ± 7.0 (8)*
	Inflamed	57.8 ± 6.9 (8)	56.6 ± 3.9 (8)‡	60.9 ± 10.0 (7)‡
Pentobarbitone, 40 mg/kg, i.p. (Days 5 and 9)	Control		46.3 ± 4.2 (9)	62.9 ± 9.8 (9)
	Inflamed		48.4 ± 5.0 (9)	62.2 ± 5.6 (9)
Pentobarbitone, 40 mg/kg, i.p. (Day 9)	Control			57.0 ± 4.2 (8)
	Inflamed			63.8 ± 5.6 (9)

† Mice were given a single injection of either mineral oil (20 µl) or *M. butyricum* (0.1 mg) in mineral oil into a foot pad and then tested with pentobarbitone 1, 5 and/or 9 days later. Values given are the mean ± s.e. mean of the number of observations in parentheses.

* Significantly different from day 1 control at $P < 0.05$; ‡ significantly different from respective control at $P < 0.05$.

polyarthritic rats. DiPasquale, Welaj & Rassaert (1974) found a dose-related response with adjuvant-induced hind paw inflammation and increased pentobarbitone sleeping times in the rat at days 2, 7 and 14.

A controversy exists concerning the effects of non-steroidal anti-inflammatory drugs on metabolic disturbances in inflammatory diseases. Zak, Honc, & Lukas (1972) reported that prolonged hexobarbitone sleeping time in polyarthritic rats was reversed by treatment with phenylbutazone, flufenamic acid and indomethacin. Other investigators have failed to observe this drug effect. For example, DiPasquale *et al.* (1974) inhibited the severity of polyarthritis with phenylbutazone but could not reverse the increased pentobarbitone sleeping time in rats. Beck & Whitehouse (1974) reported similar failure of several drugs to prevent depressed drug metabolism in arthritic rats. In the present study, inflammation in the mouse was inhibited by indomethacin (1 and 3 mg/kg, i.p.) but no effect was observed on ketamine-*M. butyricum*-induced depression of drug metabolism.

Anaesthetics and tranquillizers have been reported to induce cellular membrane expansion, fluidization and disorder of the components in the membrane (Seeman, 1972). Although *M. butyricum* itself did not cause depression of metabolism in the mouse, ketamine and pentobarbitone may have sensitized the hepatic membranes to the effect of *M. butyricum* or some 'toxohormone', as Whitehouse (1973) has speculated. Since the ketamine-*M. butyricum*-induced depression of metabolism was maximal at day 5 (the time when hind paw inflammation was maximal) and since it correlated with the severity of the inflammatory disease, the presence of a toxohormone, released during the active phase(s) of inflammation, seems reasonable. Further investigation into the nature of this rather unusual effect of anaesthetics in the presence of *M. butyricum*-induced hind paw inflammation in the mouse is necessary to explain the results fully.

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