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5-Methoxytryptophol injections in the Syrian hamster: plasma and pineal concentrations

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Plasma and pineal 5-methoxytryptophol (ML) levels were determined by radioimmunoassay (RIA) following subcutaneous (s.c.) injection of ML (1, 25 and 100 μg) to hamsters, *Mesocricetus auratus*. A dose-dependent increase in plasma ML occurred, with the duration of the supra-physiological levels being related to the dose. However, only the 100 μg dose significantly increased pineal ML levels above control values. Plasma profiles after 1 or 25 μg ML fitted a one-compartment model with half-lives of 18.6 and 25.2 min, respectively. Following 100 μg ML a biphasic decay curve was evident, the half-lives of the 2 phases being 6.6 min and 2.95 h.

The 5-methoxyindole, 5-methoxytryptophol (ML), is synthesized in the mammalian pineal gland, retina and Harderian gland [11, 16]. This indole has been measured in the pineal gland of several species (rat [2, 4, 9, 13], hamster [3, 13], cow [2], pig [2], sheep [2, 7, 13], man [1], pigeon [6], tortoise [13, 17] and turtle [15]).

In order to determine the physiological role of ML, researchers have investigated the biological activity of exogenously administered ML in a number of different experimental models (review in ref. 10). A stimulatory and inhibitory effect of ML on the gonadal axis has been reported and, in some cases, ML has been shown to be without effect. However, none of these studies have measured the ML levels in the circulation following its administration, and, to date, no information about the pharmacokinetics of ML has been reported.

In this study the radioimmunoassay (RIA) for pineal ML [13] has been adapted and validated to measure hamster plasma ML. Plasma and pineal concentrations of ML after subcutaneous (s.c.) injection of 3 different doses of ML were then determined in the animals.

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Male Syrian hamsters (*Mesocricetus auratus*) weighing 70–80 g were purchased from Centre d'Elevage du Nord, Lewaarde, France where they had been raised under a photoperiodic schedule of 14 h light (L)–10 h dark (14L:10D). In order to adapt to the laboratory conditions the animals were kept in 14L:10D (lights on from 04.00 to 18.00 h) at an ambient temperature of 20 ± 1°C for 3 weeks prior to the experiment. Food pellets and tap water were available ad libitum.

At 14.00 h the hamsters were injected s.c. with 1, 25 or 100 μg ML or control vehicle (1% ethanol in distilled water). At 15, 30, 60, 120 and 240 min after injection the animals ($n = 5$ per time point) were killed, the pineal glands quickly removed, placed in 1 ml tricine buffer (0.1 M, pH 5, containing 0.9% NaCl and 0.1% gelatine), frozen in liquid nitrogen and stored at −20°C until analysis. Trunk blood samples were centrifuged and the plasma was kept at −20°C until assay.

The pineals were sonicated and ML was measured in the homogenate by a RIA which had previously been validated to measure hamster pineal ML [13]. The sheep antiserum (Batch 1320) was used at a final dilution of 1:32,000. The major cross-reacting substances were 5-methoxyindole (0.73%), *O*-acetyl-5-methoxytryptophol (0.45%) and melatonin (0.10%).

In this study the RIA was also validated for the direct measurement of ML in hamster plasma. For the standard curve pooled heparinized hamster plasma was charcoal-stripped to remove ML. The plasma was mixed with charcoal (10%) for 2 h at 4°C, and then separated by centrifugation (40,000 *g* for 30 min) and filtration (0.45 μm, Sartorius filters). Parallelism, recovery and extraction studies were performed.

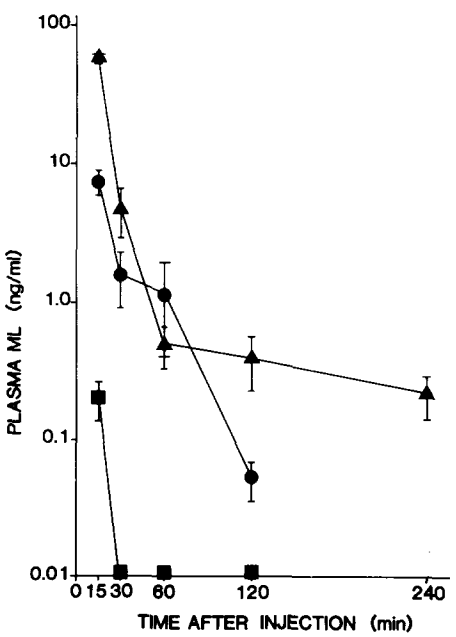

Fig. 1. Plasma 5-methoxytryptophol (ML) levels in hamsters following subcutaneous injection of 1 (■), 25 (●) and 100 (▲) μg ML at 14.00 h. Values are means ± S.E.M., $n = 5$.

Plasma samples (600 μl) were extracted with 6 ml dichloromethane and a 5-ml aliquot was evaporated to dryness under vacuum. The residue was reconstituted in 500 μl tricine buffer and assayed.

Regression lines were fitted to the $\log_{10}$ concentration versus time graphs. The half-life was calculated from the slope of the line. Comparison between 2 groups (ML-injected and vehicle-injected animals) at a particular time point was performed using the unpaired Student's t-test.

The sensitivity of the assay was 1 pg/tube (5 pg/pineal, 5 pg/ml plasma). Pooled hamster plasma serially diluted with charcoal-stripped plasma gave displacement parallel to that of ML standards serially diluted with charcoal-stripped plasma. A plasma ML standard curve extracted with dichloromethane was superimposable on the buffer ML standard curve. Recovery of ML (5 and 20 pg) from hamster plasma was 96 ± 8 and $122 \pm 3\%$ (mean $\pm$ S.E.M., $n = 6$ each), respectively.

Following s.c. injection of ML there was a dose-dependent increase in ML in the plasma (Fig. 1). After 15 min plasma concentrations (mean $\pm$ S.E.M.) were 57.5 ± 4.2, 7.3 ± 1.7 and 0.20 ± 0.07 ng/ml for 100, 25 and 1 μg ML, respectively. Vehicle-injected controls killed at the same time (15 min post-injection) had significantly lower plasma ML levels ($P < 0.05$ for 1 and 25 μg injection, $P < 0.001$ for 100 μg injection). Within 30 min plasma ML levels after 1 μg ML were similar to control values whereas after 2 h (25 μg) and 4 h (100 μg) the mean plasma ML concentrations were still above control values (25 μg controls 0.029 ± 0.021 ng/ml, ML group 0.058 ± 0.013 ng/ml; 100 μg controls 0.024 ± 0.009 ng/ml, ML group 0.214 ± 0.074 ng/ml). However, the difference between these control and ML groups was not significant.

Plasma profiles following 1 or 25 μg ML fitted a one-compartment model with half-lives of 18.6 and 25.2 min, respectively. After administration of 100 μg a biphasic decay curve was observed, the half-lives of the 2 phases being 6.6 min and 2.95 h. Areas under the plasma concentration curve were 29, 4526 and 8863 pg/ml·h for 1, 25 and 100 μg, respectively.

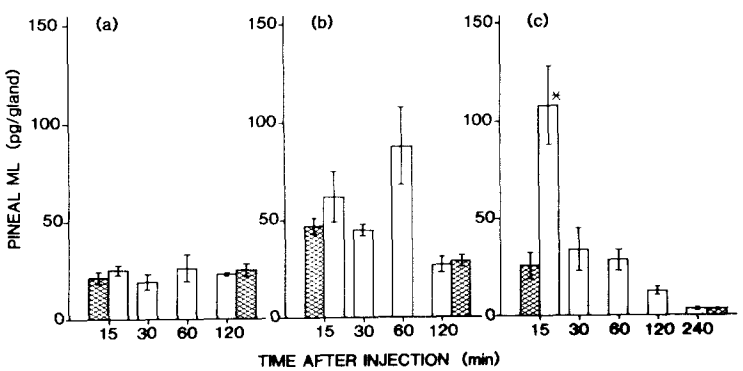

Fig. 2. 5-Methoxytryptophol (ML) levels in hamster pineal glands following subcutaneous injection of (a) 1, (b) 25 and (c) 100 μg ML at 14.00 h. Values are means $\pm$ S.E.M. ($n = 5$) of ML-injected (open column) and vehicle-injected (hatched column) animals. $*P < 0.02$ compared with control group at 15 min post-injection.

The amount of ML in the hamster pineal gland was also determined following injection of ML or vehicle (Fig. 2). After 15 min there was a significant increase in pineal ML following 100 μg ML compared to the control group ($P<0.02$). On the other hand, pineal ML levels following 1 and 25 μg ML were similar to those following injection of vehicle.

A rhythm in pineal ML has been previously demonstrated in Syrian hamsters kept in a 14L:10D photoperiod [14]. Daytime levels were 81 ± 7 pg/gland ($n=37$) which declined to 15 ± 4 pg/gland ($n=10$) during darkness. Peak levels occurred between 11.45 and 13.45 h. An injection time of 14.00 h was chosen for this study since this corresponds to a time when pineal ML levels are on a declining peak and thus false increases due to endogenous ML could be avoided.

This study in the hamster demonstrates that plasma ML levels following exogenous ML administration decrease rapidly during the post-injection period. Subcutaneous injection of 25 μg melatonin to Syrian hamsters produced plasma melatonin levels in excess of 100 ng/ml 15 min post-injection [12]. A similar dose of ML in our study produced plasma ML levels of 10- to 20-fold less.

The dramatic decline in plasma ML during the post-injection period could be a result of rapid distribution or elimination (metabolism and excretion). The primary route of metabolism of ML in rats is oxidation into 5-methoxyindole acetic acid [5, 8] but the rate at which this reaction occurs is not known. The relatively low levels of ML measured in the pineal gland following ML administration suggest that, at least in the hamster pineal, ML is not actively taken up and stored.

Plasma levels after injection of 25 μg ML fitted a one-compartment model whereas after a 100 μg ML injection plasma levels fitted a two-compartment model. A rapidly declining first phase following 25 μg ML may have been missed under our experimental conditions. On the other hand this finding could indicate the presence of a more slowly equilibrating compartment following the higher dose of ML, such as increased uptake and storage of ML in other tissues or saturation of its metabolizing enzymes. This change in pharmacokinetic parameters with an increasing dose of ML should be borne in mind when experiments involving exogenously administered ML are interpreted.

In conclusion, there was a dose-dependent increase in plasma ML following s.c. injection of 1, 25 and 100 μg ML to hamsters, duration of the supra-physiological plasma levels being proportional to the dose.

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