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Effects of cold stress and ether stress on aminopyrine demethylation kinetics in vivo

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The effect of cold stress and ether stress on mixed function oxidase activity in vivo has been studied using the aminopyrine–$^{14}CO_2$ exhalation rate (CER) method. Cold stress following both acute and chronic exposure resulted in enhanced rate of demethylation of both aminopyrine (CER α half-life) and its monomethyl metabolite (CER β half-life). However the time course of the response to cold stress differs for the two demethylations. The characteristics of the cold-induced enhancement of aminopyrine-CER would appear to differ in several aspects from that reported for phenobarbitone pretreatment. In contrast, ether exposure did not produce any consistent effect on drug metabolizing status of the rat.

The determination of hepatic mixed function oxidase activity in vivo under conditions of environmental stress requires a judicious experimental protocol. The methodology employed must not induce trauma in itself and hence the use of anaesthesia, surgery or other invasive techniques is precluded. One means of assessing drug metabolizing status under such restrictions involves the monitoring of volatile metabolites in expiratory air after administration of a drug with a suitably radiolabelled, labile moiety.

Lauterburg & Bircher (1976) first demonstrated that $^{14}CO_2$ exhalation rate (CER)–time profiles following administration of [*N*-dimethyl-^{14}C]aminopyrine to the rat was a good in vivo index of hepatic mixed function oxidase activity. Other investigators have also used this approach (Sultatos et al 1978; Willson et al 1979). Recently we have refined the aminopyrine-CER method by using an extended time to fully characterize the time profile (Houston et al 1981). Under these conditions the CER declines in a biphasic fashion which is consistent with the fact that the two [*N*-methyl-^{14}C] groups are cleaved from the aminopyrine molecule at different rates. The plasma half-life of the monomethyl metabolite is 2–4 times longer than the plasma half-life of aminopyrine (Lockwood & Houston 1980). Curve stripping of the CER-time profile yields a fast α-phase half-life, which reflects the aminopyrine plasma half-life, and a slower β-phase half-life, which reflects the monomethyl metabolite half-life. The sensitivity of the aminopyrine-CER method to perturbations in drug metabolizing status is increased by the use of a cross-over experimental design (Houston et al 1981).

Animals exposed to both low environmental temperatures and ether vapour show the classic stress response. Circulating corticosterone concentrations are elevated (Maickel et al 1963; Kendell et al 1965) and increased release of other hormones (Matsuda et al 1964; Docommun et al 1966; Muller et al 1967) and catecholamines (Leduc 1961; Vellucci 1977) occurs. The consequences of cold stress but not ether stress on drug metabolism has been studied in vitro. Different groups of investigators have demonstrated enhanced microsomal enzyme activity (Inscoe & Axelrod 1960; Stitzel & Furner 1967; Stitzel & McCarthy 1972; Fuller et al 1972). Accelerated rates of metabolism have also been reported using isolated perfused livers from cold exposed rats (Fuller et al 1972). However, the consequences of cold stress may not be manifest with all drug metabolic pathways (Inscoe & Axelrod 1960; Furner & Stitzel 1968). Also the nature of the response to cold and other stresses may vary from one rat strain to another (Stitzel & McCarthy 1972).

We have investigated the effect of acute and chronic (6 day) cold exposure on the drug metabolizing status of the rat in vivo to supplement the previous work in this area. Also we have carried out preliminary studies on the consequences of a milder form of environmental stress-ether exposure. The model compound selected for use was aminopyrine since this drug is slowly cleared (Lockwood & Houston 1979) and shows minimal protein binding in plasma and tissues (Brodie & Axelrod 1950). Thus changes in aminopyrine clearance are accurately

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reflected by charges in its half-life (Perrier & Gibaldi 1974) and the CER method is a valid procedure to assess drug metabolizing status.

MATERIALS AND METHODS

Chemicals

Aminopyrine and acetylaminopyrine were obtained from Aldrich Chemical Co Ltd, aminoantipyrine and antipyrine from BDH Chemicals Ltd. [*N*-dimethyl-^{14}C]aminopyrine supplied by the Radiochemical Centre, Amersham, had a specific activity of 25 mCi mmol^{-1} and a radiochemical purity of 99% as determined by t.l.c.

Animals, housing and treatment

Male Wistar rats (210–260 g) were housed individually. Feed and bedding were constant throughout the study. During control periods, animals were maintained at 22 °C in a thermostatically controlled room. During periods of cold stress animals were housed in a walk-in cold room maintained at a nominal temperature of 4 °C.

Immediately following [*N*-dimethyl-^{14}C]aminopyrine administration by the intraperitoreal route each rat was placed in an individual metabolism cage. The $^{14}CO_2$ exhalation rate was determined over 6 h and urine collected over 24 h.

Acute cold stress experiments were commenced by administering aminopyrine at a time corresponding to the initial exposure to the cold environment. After 24 h the animals were removed from the cold environment and were returned to the 22 °C room. Chronic cold stress studies were carried out by housing the animals in the cold room for five days before the final CER study. At the start of the sixth day at 4 °C aminopyrine was administered. Control studies were carried out at room temperature (22 °C).

In the ether exposure studies, a constant, subanaesthetic concentration of ether was maintained in the metabolism cage. This was achieved by introducing a pair of glass two-way taps and a bubble trap containing ether into the inflowing air tubing. By means of the taps the airflow down the main tube could be intermittently redirected through the ether vessel. By limiting the period of ether administration to 20 s every 20 min an ether concentration could be maintained which caused obvious agitation without anaesthesia. A cross-over design was adopted for acute stress studies allowing one week between aminopyrine tests. The effect of chronic cold exposure was determined by a longitudinal design following on from the second aminopyrine test.

Determination of $^{14}CO_2$ exhalation rates

Each rat was housed in an individual all-glass metabolism cage adapted for CO_2 collection. Air was drawn through the system by means of an electric pump. A soda lime trap removed endogenous CO_2 from the air stream before passing through the metabolism cage. The air was then bubbled through a concentrated sulphuric acid trap (20 ml) and two CO_2 traps (each contained 40 ml of ethanolamine: methanol, 1:4) in series. The CO_2 trapping fluid in the first trap was changed every 10 min for the first 2 h and then every 20 min for a total of 6 h. The second trap was changed periodically to ensure that all of the $^{14}CO_2$ had been collected in the first trap. Aliquots of trapping fluid were assayed for radioactivity in Instagel (Packard Co Ltd) using a Packard Tricarb Scintillation Counter (Model 2405) with internal standard quench correction.

Half-lives associated with the decline in $^{14}CO_2$ exhalation with time were calculated by plotting the logarithm of the $^{14}CO_2$ exhalation rate against the mid-point of the time interval. The terminal (β-) half-lives were calculated by method of least squares. The faster (α-) half-lives were obtained by curve stripping and the best line of fit for these residual points also calculated by the least squares principle. The total percentage dose exhaled as $^{14}CO_2$ was calculated by summing the observed $^{14}CO_2$ output over the experimental period and the extrapolated $^{14}CO_2$ output from the end of experimental period to infinity. The extrapolated value was obtained by dividing last exhalation rate by β. The maximum $^{14}CO_2$ exhalation rates reported refer to maximum observed values.

Determination of urinary excretion products

An aliquot of diluted urine was basified and an internal standard (antipyrine) added. This mixture was extracted into chloroform (phase volume ratio 1:5). The organic layer was then removed to a clean test tube, evaporated to dryness under nitrogen and reconstituted in methanol. The aminopyrine, monomethylaminoantipyrine, aminoantipyrine and acetylaminoantipyrine concentration in the methanol solution was quantified by reverse-phase h.p.l.c. as described previously (Lockwood & Houston 1979). The total radioactivity in each urine was also assayed.

RESULTS

Typical biphasic CER-time profiles from an individual rat from the cold exposure studies are shown in Fig. 1. The four parameters which characterize the

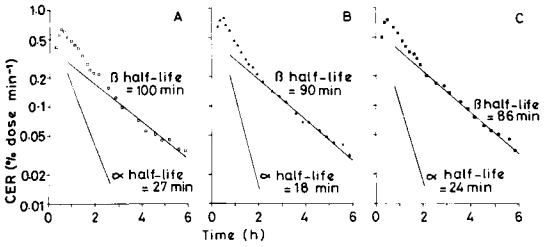

FIG. 1. $^{14}CO_2$ exhalation rate (CER)-time profiles in an individual rat following administration of [*N*-dimethyl-^{14}C]aminopyrine under control conditions (panel A), during acute cold stress (panel B) and during chronic cold stress (panel C).

CER-time profile (α-half-life, β-half-life, maximum CER and cumulative $^{14}CO_2$) for each of the rats studied under control and stress conditions are illustrated in Fig. 2. It can be seen that a decrease in the α-half-lives occurs during acute cold exposure. However if cold exposure becomes chronic, there is a tendency for the α-half-life to increase relative to the acute situation. Six of the β-half-lives decreased during acute cold and four were further decreased on chronic exposure. One of the seven rats studied was not influenced by cold stress. Both half-lives were statistically signficantly reduced during acute and chronic cold exposure (Table 1). Cumulative $^{14}CO_2$ was statistically significantly reduced during the acute stress. Changes in this parameter after chronic stress were less marked and did not achieve statistical significance. No trends were apparent in the maximum CER changes observed (see Fig. 2).

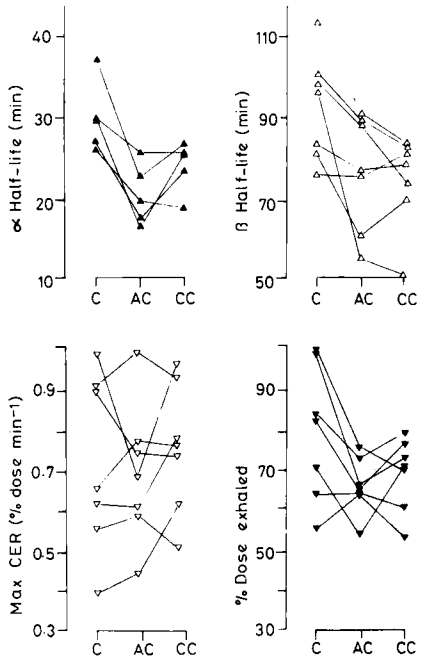

FIG. 2. $^{14}CO_2$ exhalation rate (CER) parameters in 7 rats following administration of [*N*-dimethyl-^{14}C]aminopyrine under control conditions (C), during acute cold stress (AC) and during chronic cold stress (CC). In 2 animals the α-phase could not be adequately characterized.

Table 1. CER parameters from rats receiving [^{14}C]aminopyrine under control conditions and following acute and chronic cold stress.

Parameter	Control	Acute cold stress	P^c	Chronic cold stress	P^c
$\alpha t_{1/2}$(min)[a]	30·0 ±4·3	20·8 ±3·7	<0·025	24·4 ±3·2	<0·025
$\beta t_{1/2}$(min)[b]	93·6 ±12·9	78·4 ±14·2	<0·01	76·0 ±11·9	<0·025
Max CER (% dose min^{-1})[b]	0·174 ±0·220	0·694 ±0·180	NS	0·758 ±0·163	NS
Cumulative $^{14}CO_2$ (% dose)[b]	78·5 ±16·8	66·7 ±6·7	<0·05	69·6 ±9·1	NS

[a] Mean of 5 animals ± standard deviation.
[b] Mean of 7 animals ± standard deviation.
[c] By 2 way analysis of variance, NS—not significant.

Ether exposure caused no statistically significant changes in either of the four CER parameters (Table 2). Fig. 3 shows the CER-time profile in a typical rat under control and during ether exposure.

The urine collected over 24 h after aminopyrine administration from each rat was analysed for parent drug and metabolites which arise from demethylation (monomethylaminoantipyrine and aminoantipyrine) and subsequent acetylation (acetylaminoantipyrine). Inter-animal variability was large under both control and stress conditions (for example see Fig. 4B for acetylaminoantipyrine). No statistically significant effects were apparent in either stress state. Under cold stress the enhanced rate of metabolism would be expected to be accompanied by a decrease in the excretion of unchanged drug. However this effect was only manifest in animals who eliminated more than 5% of the dose by renal excretion under control conditions (Fig. 4A). In the animals with low basal renal excretion this parameter was not influenced by cold stress.

Table 2. CER parameters from rats receiving [^{14}C]aminopyrine under control conditions and following ether exposure.

Parameter	Control[a]	Ether exposure[a]	P^b
$\alpha t_{1/2}$(min)	26·6 ± 8·6	23·8 ± 7·1	NS
$\beta t_{1/2}$(min)	83·2 ± 24·2	81·0 ± 19·1	NS
Max CER (% dose min^{-1})	0·670 ± 0·238	0·558 ± 0·148	NS
Cumulative $^{14}CO_2$ (% dose)	60·0 ± 19·4	59·2 ± 15·0	NS

[a] Mean of 6 animals ± standard deviation.
[b] By paired *t*-test, NS—not significant.

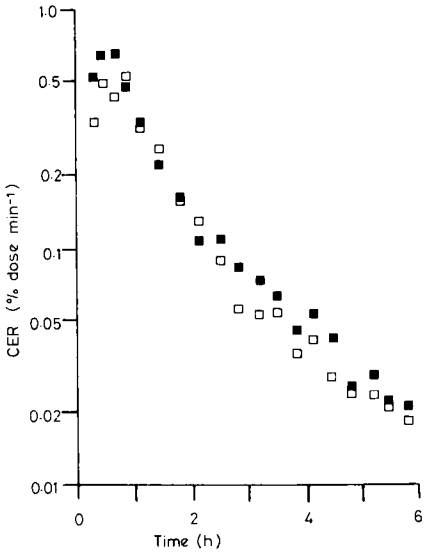

FIG. 3. $^{14}CO_2$ exhalation rate (CER)–time profiles in an individual rat following administration of [*N*-dimethyl-^{14}C]aminopyrine under control conditions (□) and during ether exposure (■).

DISCUSSION

The use of the aminopyrine-CER method has demonstrated that cold exposure results in an enhancement of hepatic mixed functions oxidase activity in vivo. This finding confirms the earlier investigations (Inscoe & Axelrod 1960; Stitzel & Furner 1967; Furner & Stitzel 1968; Stitzel & McCarthy 1972; Fuller et al 1972) carried out with microsomal preparations from rats under cold stress.

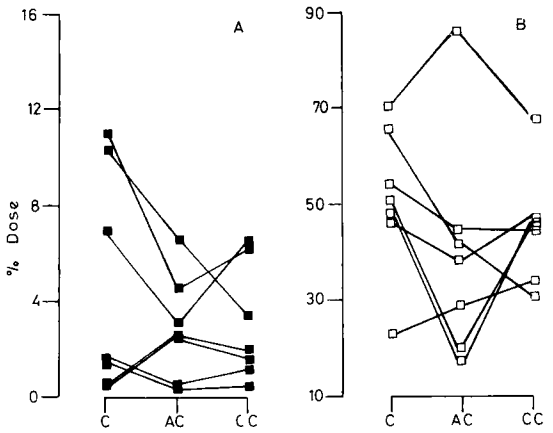

FIG. 4. Amounts of aminopyrine (panel A) and acetylamino-antipyrine (panel B) excreted in urine of 7 rats following administration of [*N*-dimethyl-^{14}C]aminopyrine under control conditions (C), during acute cold stress (AC) and during chronic cold stress (CC).

On the assumption that the demethylation of aminopyrine is accurately reflected in the CER α-phase and that the CER β-phase represents demethylation of its monomethyl metabolite cold exposure promotes both reactions. However the time course of this stress response would appear to differ for the two demethylations. During acute cold exposure the β:α half-life ratio increases from a control value of 3·1 ± 0·9 to 4·1 ± 1·0 ($P < 0.01$ by paired *t*-test). A reversal of this trend is observed under chronic cold exposure when the ratio (3·4 ± 0·4) is not significantly different from control. This would suggest that the promotion of mixed function oxidase activity by cold stress is apparent earlier with the initial demethylation of aminopyrine than with the subsequent demethylation of the monomethyl metabolite.

Table 3. Urinary excretion products and urinary radioactivity obtained from rats receiving [^{14}C]aminopyrine under control conditions and following exposure to cold or ether.

	Urinary excretion products (% dose)				
	Amino-pyrine	Mono-methyl-amino-anti-pyrine	Amino-anti-pyrine	Acetyl-amino-anti-pyrine	Urinary radio-activity (% dose)
Cold stress study					
Control	6·2 ± 5·0	1·7 ± 1·2	10·2 ± 6·6	51·9 ± 15·4	18·6 ± 5·9
Acute cold	2·7 ± 2·3	1·4 ± 1·1	9·4 ± 6·3	39·8 ± 23·4	21·5 ± 2·5
Chronic cold	4·3 ± 3·5	1·2 ± 0·8	8·3 ± 6·3	45·4 ± 12·4	21·0 ± 3·5
Ether stress study					
Control	2·1 ± 2·6	2·5 ± 3·4	11·7 ± 5·8	60·8 ± 12·3	18·4 ± 6·3
Ether exposure	3·4 ± 3·5	2·6 ± 3·5	13·4 ± 4·9	65·1 ± 19·9	19·3 ± 5·6

Mean of 6–7 rats ± standard deviation. No excretion product is statistically significantly different when control and stress urines are subjected to a 2-way analysis of variance (cold stress) and a paired *t*-test (ether stress).

It would be predicted that a reduction in the CER half-life would produce an increase in the maximum CER observed. This does not occur during cold exposure because the cumulative $^{14}CO_2$ produced and hence the area under the CER-time curve is reduced by this stress. It would appear that the alternative pathway(s) to demethylation responsible for the metabolism of aminopyrine are preferentially enhanced under cold stress. It is of interest to compare this behaviour with that reported following phenobarbitone induction in the same aminopyrine-CER system (Houston et al 1981). Phenobarbitone pretreatment reduced the CER half-lives by a similar degree to cold stress. In contrast to the present studies the cumulative $^{14}CO_2$ produced is not altered

and therefore the maximum CER observed is increased following phenobarbitone pretreatment.

The increased rate of metabolism of aminopyrine which is observed during cold exposure results in a decrease in the renal excretion of parent drug. In agreement with the previous studies using phenobarbitone induction this effect is only apparent in animals that may be described as comparatively high renal excretors (that is eliminate more than 5% of the aminopyrine dose unchanged under control conditions). A notable characteristic of phenobarbitone induction of aminopyrine metabolism is the decrease in the acetylaminoantipyrine recovered in the urine (Houston et al 1981). In cold stress this urinary excretion product is unaltered (see Fig. 4B). Thus the effects of cold exposure and phenobarbitone pretreatment on the activity of the mixed function oxidase as measured by the aminopyrine-CER method differ in certain aspects. Similar conclusions were drawn by Stitzel & Furner (1967, 1968) who compared both types of enhancements using in vitro systems.

The contrast between the results obtained during cold and ether stress is perhaps not surprising. Although both stressors are documented to alter hormonal status, ether exposure may not be sufficiently stressful to elicit the same response as cold treatment. Microsomal studies have also documented that changes in drug metabolism can be dependent upon the nature of the stress treatment (Stitzel & McCarthy 1972). Furthermore, recent studies (Aune et al 1981; Johannessen et al 1981) have demonstrated that ether can directly inhibit hepatic mixed function oxidase activity when administered at anaesthetic doses. In the present experiments although care was taken to maintain ether exposure below that of anaesthesia, some degree of inhibition may have occurred. Thus the observed lack of response may reflect the net effect of direct inhibition and indirect, stress-mediated enhancement of mixed function oxidase activity by ether.

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