Some factors modifying the metabolism of AH 8165 by rat liver homogenate *in vitro*

AH 8165 [1,1'-azo bis (3-methyl-2-phenyl-1H-imidazo (1,2a) pyridinium)dibromide] is a rapidly acting non-depolarizing neuromuscular blocking agent in both man (Simpson, Strunin & others, 1972) and experimental animals (Bolger, Brittain & others, 1972).

Animal experiments both *in vivo* and *in vitro* show that AH 8165 is extensively metabolized to inactive metabolites (Bolger & others, 1972), although in man at least 70 to 80% is excreted unchanged (Blogg, 1973). However, in animal experiments *in vitro*, AH 8165 has been shown to be metabolized under anaerobic conditions by an NADPH-dependent azoreductase present in liver microsomes (Bolger & others, 1972).

Since disappearance of neuromuscular blocking activity is readily measured biologically, it was considered possible that the rate of deactivation of AH 8165 in liver homogenates could be used as a measure of reductase enzyme activity.

In the following preliminary study the effects of various factors on the capacity of the rat liver to degrade AH 8165 have been studied *in vitro*. The rat phrenic nerve diaphragm preparation (Bulbring, 1946) was set up at 37° in Krebs solution, gassed with 5% carbon dioxide in oxygen. The phrenic nerve was stimulated at supramaximal voltage, 0·1 ms pulse width and a frequency of 0·2 Hz. The response to a given concentration of neuromuscular blocking agent was measured as the % inhibition of twitch height after 5 min contact time. A 15 min dose cycle was used.

10% rat liver homogenates were prepared in cold (+ 4°) 0·25M phosphate buffer (pH 7·3) using an Ultra Turrax homogenizer. The livers were obtained from female albino Wistar rats (250–300 g) which were killed by a blow on the head. Liver homogenates, once prepared, were used immediately.

A standard concentration effect curve for either (+)-tubocurarine or AH 8165, was obtained by adding various concentrations of the drug to the assay tissue at the same time as an aliquot of homogenate containing no drug. The mean (± standard error) concentration of AH 8165 causing 50% depression of twitch height was 10·1 ± 0·4 μg ml^{-1} (n = 46) and for (+)-tubocurarine 1·1 ± 0·1 μg ml^{-1} (n = 7).

Either (+)-tubocurarine or AH 8165 was added to rat liver homogenate incubated at 37° and bubbled with either oxygen or nitrogen. Aliquots of homogenate were selected so that the bath concentration for the assay tissue was nominally 20 μg ml^{-1} AH 8165 or 2 μg ml^{-1} (+)-tubocurarine. These concentrations produced 80 to 90% inhibition of twitch height. Aliquots of the homogenate containing drug were added to the assay tissue after 0, 5, 10, 15, 30 and 45 min incubation. The percentage inhibition of twitch height obtained with these aliquots was compared with the standard concentration effect curve for the drug. The results for the incubated drug were expressed as the percentage of the initial concentration of the drug remaining in the homogenate.

The activity of (+)-tubocurarine was not reduced by incubation in rat liver homogenate, but that of AH 8165 was reduced. The rate of deactivation of AH 8165 was greatest when the initial concentration of AH 8165 was 50 to 125 μg ml,$^{-1}$ and the homogenate was bubbled with nitrogen. All subsequent experiments were carried out using these optimum conditions. The results were plotted as log % AH 8165 remaining in the homogenate against time. A linear relation was obtained and regression lines were calculated. From these lines, half times (i.e. time until 50% of the drug remains), for AH 8165 in liver homogenate were obtained.

The liver donating rats were subjected to a number of pretreatment courses and the effects of these on the half time of AH 8165 in the liver homogenate are shown in Table 1.

Table 1. *Factors modifying the metabolism of AH 8165 by rat liver homogenates.*

Pretreatment of liver donating rats	AH 8165 half time	95% Confidence interval
Control	14 min 5 s	9 min 24 s to 18 min 48 s
Starved 24 h	4 min 48 s	2 min 6 s to 7 min 36 s
Phenobarbitone 60 mg kg^{-1} daily for 4 days	5 min 6 s	3 min 36 s to 6 min 36 s
SKF 525A 15 mg kg^{-1} 45 min pretreatment	29 min 59 s	15 min 6 s to 44 min 48 s
Diazepam 5 mg kg^{-1} twice daily for 4 days	6 min 18 s	4 min 30 s to 8 min 0s

All drugs were administered intraperitoneally.

The pretreatment courses used were those with documented inductive or inhibitor activity towards other drug metabolizing enzymes. Phenobarbitone induces the activity of a number of enzyme systems, particularly the mixed function oxidase enzymes and SKF 525A inhibits a large number of drug metabolizing enzymes (Burger, 1968). Diazepam has been shown to increase the rate of metabolism of pentobarbitone (Jori, Prestini & Pugliatti, 1969) and dimethylaminobenzene reductase is markedly induced by short periods of starvation (Jervell, Christoffersen & Mörland, 1965). In all cases these pretreatment courses had similar effects upon the degradation of AH 8165 in rat liver homogenate as those reported on other enzyme systems.

Thus these initial experiments indicate that the metabolism of AH 8165 by rat liver homogenates *in vitro* may be a useful and simple screen for enzyme inducing and inhibiting agents.

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