The Interaction between Pilocarpine and Hexobarbital in Male Rats

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Abstract. The interaction between pilocarpine and hexobarbital was studied in male rats. Hexobarbital was infused continuously. The dose needed to obtain an EEG criterion (the "silent second") was determined. The ensuing anesthesia times after these equi-anesthetic doses were also recorded. At different times prior to the hexobarbital threshold determination the rats were pretreated with 25–200 mg/kg of pilocarpine. In most experimental series pretreatment with methylatropine (2 mg/kg s.c.) was also given to reduce the effects of pilocarpine on peripheral cholinergic sites.

In the dose-response study pilocarpine was given 1 h prior to the hexobarbital threshold determination. Pilocarpine in doses of 25–50 mg/kg increased the amount of hexobarbital needed to obtain the "silent second". With higher doses of pilocarpine, increases in hexobarbital thresholds were seen if no convolution had been induced by the pilocarpine treatment. If a convolution was recorded the dose of hexobarbital was reduced. Similar results were obtained in the time-effect studies where more convulsions tended to appear if the time between the dose of pilocarpine and the dose of hexobarbital was increased. In animals without convulsions the effect of pilocarpine on the dose of hexobarbital was counteracted by atropine (8 mg/kg i.p.). The ensuing anesthesia times were increased in the pilocarpine pretreated animals, which could be due to either the pilocarpine dose, the increased dose of hexobarbital needed to obtain the "silent second", or both. No regression between body temperature and dose of hexobarbital was found, but there was a regression with the ensuing anesthesia times.

The effects of pilocarpine with an increase in hexobarbital threshold is similar to the changes seen in the threshold in the abstinence after chronic barbital treatments. More important, however, is that both increases are reduced by convulsions. Could pilocarpine be a model for the changes in the abstinence after barbital?

Key words: Pilocarpine — Hexobarbital — Rats and drug interaction.

INTRODUCTION

Increased activity in cholinergic systems in the central nervous system (CNS) has been implicated as one possible explanation for the tolerance seen after chronic barbiturate treatments (Ekwall and Wahlström, 1972; Wahlström and Ekwall, 1976). The tolerance was in these studies tested with an EEG-threshold method where hexobarbital was infused in the tail vein of male rats (Wahlström, 1966a). In this connection it was of interest to have a clear picture of the interaction between manipulations of central cholinergic mechanisms and barbiturates in acute experiments.

Earlier studies of the interaction between drugs acting on cholinergic mechanisms in the CNS and CNS depressants have utilized the anesthesia times. No clear picture has emerged from these studies, especially after cholinergic stimulation (Proctor, 1965; Kayaalp and Numanoglu, 1965; Barnes and Meyers, 1964; Davis et al., 1971). Since the hexobarbital threshold is much less influenced by extracerebral factors which might influence the anesthesia times it was decided to reinvestigate the interaction between cholinergic mechanisms in the CNS and depressants using the hexobarbital threshold method. The results from a study of cholinergic blockade by atropine is reported elsewhere (Wahlström, 1976). The present paper deals with the effects of cholinergic stimulation through pilocarpine on the hexobarbital threshold. When it was found that some of the increased varia-
bility, seen after acute pretreatment with pilocarpine, was dependent on whether a convulsion was induced or not, the relevance of the present results for the tolerance studies with a similar interest in convulsions (Wahlström, 1973, 1975a) was increased. Some of the results have been briefly presented elsewhere (Wahlström, 1975b).

METHODS

Male Sprague-Dawley rats (Nih/Han/Mol, Møllegaard, Li. Skensved, Denmark) with initial body weights around 300 g were used. They were kept 3/cage in an animal room with a temperature of approximately 26°C. The room was excluded from all external lights. The artificial light in the room was on between 20:00 and 08:00. Food and drinking fluid was available ad lib.

The anesthesia threshold method has been described in detail elsewhere (Wahlström, 1966a). Sodium hexobarbital is infused in a tail vein with a constant rate of 0.25 mg/kg/s (volume rate 0.1 ml/min). The electroencephalogram is recorded during the infusion. The first burst suppression of 1 s or more is used as the threshold criterion (the "silent second"). The dose of hexobarbital needed to obtain this "silent second" was determined. Since the different animals tend to have individual threshold doses the results are usually given as a percentage of a preexperimental average calculated on two or three threshold determinations performed prior to any experimental series. Two groups of animals designated experiment No. 1 and experiment No. 2 were included in the present material. The pre-experimental average of threshold dose was in all rats in experiment No. 1 65 ± 1.4 (± S.E.) mg/kg and in all rats in experiment No. 2 68 ± 1.6 mg/kg. All threshold determinations were performed during the first 5 h of the activity period (darkness) of the rat.

Immediately after the "silent second" the infusion was stopped. The ensuing anesthesia times were recorded on automatic beds in a constant temperature room (30 °C) as described in detail elsewhere (Wahlström, 1966b). When the reflexes returned the rats turned over, fell from the beds, and the recording was stopped. To ensure that all animals received equi-anesthetic doses of hexobarbital at preexperiments times where more than 7.5 mg/kg of hexobarbital had been infused after the "silent second" were excluded. The average pre-experimental values of ensuing anesthesia times were in experiment No. 1 12 ± 1 min and in experiment No. 2 14 ± 1 min.

The rectal temperature was recorded with a thermistor probe as one part of a Wheatstone bridge. The probe was inserted 5 cm in the rectum. All temperature recordings were performed immediately prior to the threshold determinations.

The occurrence of convulsions were either observed or recorded in jiggle cages where the animals were housed individually after the injection of pilocarpine. The jiggle cages were suspended below a horizontal metal tongue. The activity pattern was picked up by the recording system and differentiated from normal activity. Observations after administration of convulsive substances it was in preliminary tests ascertained that convulsive episodes could be picked up by the recording system and differentiated from normal activity.

The following intraperitoneal doses of pilocarpine and time intervals between dose of pilocarpine and threshold determinations were used in the different consecutive series of experiments performed on the rats included in experiment No.1:

Series No. 1: 25 mg/kg, 1 h. Series No. 2: 50 mg/kg, 1 h. Series No. 3: 100 mg/kg, 1 h. Series No. 4: 50 mg/kg, 0.5 h. Series No. 5: 50 mg/kg, 2 h. Series No. 6: 50 mg/kg, 4 h, and Series No. 7: 100 mg/kg, 1 h (similar to series No. 3).

Included in Experiment No. 2 were:

Series No. 8: 100 mg/kg, 1 h. Series No. 9: 200 mg/kg, 1 h. Series No. 10: 100 mg/kg, 1 h and Series No. 11: 50 mg/kg, 1 h.

Series Nos. 8 and 9 were performed as a cross-over experiment. In series Nos. 10 and 11 8 mg/kg of atropine was given intraperitoneally 0.5 h prior to the pilocarpine dose.

One hour prior to all doses of pilocarpine except in series Nos. 10 and 11 2 mg/kg of methylatropine was given subcutaneously. Control runs with only methylatropine were performed prior to all series except series No. 7 in experiment No. 1. In Experiment No. 2 a control run without pretreatment was done between the two parts of the crossover design of series Nos. 8 and 9. A control run with only 8 mg/kg of atropine was performed between series Nos. 10 and 11. There was at least a week between two treatments with pilocarpine in the same rat. If not otherwise stated two tailed Student's t-test was used to test the differences between active treatments and corresponding controls.

The following abbreviations were used: S.E. = standard error of the mean, N = number, r = correlation coefficient, b = linear regression coefficient, df = degrees of freedom, CNS = central nervous system.

All doses are given as the salts. The following substances were used: sodium hexobarbital, pilocarpine chloride, atropine sulphate, and methylatropine nitrate.

RESULTS

The results of the dose-response studies with pilocarpine are seen in Figure 1A. All doses were given 1 h prior to the hexobarbital threshold determination. The dose of 25 mg/kg had a slight effect seen as an

![Fig. 1. Dose-response (A) and time-response (B) curves for effect of pilocarpine on dose of hexobarbital needed to obtain a "silent second". Dose of hexobarbital given in percent of a pre-experimental average. All animals pretreated with methylatropine 2 mg/kg s.c. 1 h prior to pilocarpine. Corresponding dose was given in the control runs. (A) Following series (filled symbols) and corresponding control run (unfilled symbol) were used: Series No. 1 (25 mg/kg), series No. 2 (50 mg/kg), series No. 3 (100 mg/kg), and series No. 9 (200 mg/kg). Circle denotes series from Experiment 1, square denotes series from Experiment 2. Number of animals was 11—13. Standard error of mean indicated by bar. (B) Following series (filled symbols) and corresponding control run (unfilled symbol) were used: Series No. 4 (0.5 h), series No. 2 (1 h), series No. 5 (2 h), and series No. 6 (4 h). In all series 50 mg/kg pilocarpine was given i.p. Number of animals was 7—12. Standard error of mean indicated by bar.](image-url)