CHRONOBIOLOGY & CHRONOMEDICINE

BASIC RESEARCH AND APPLICATIONS

EDITED BY
G. HILDEBRANDT / R. MOOG / F. RASCHKE

Verlag Peter Lang
Frankfurt am Main · Bern · New York · Paris

CIP-Kurztitelaufnahme der Deutschen Bibliothek

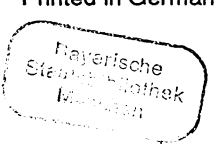
Chronobiology & [and] chronomedicine : basic re= search and applications ; Marburg/Lahn, FRG, 1986 / [Europ. Soc. for Chronobiology. Ed. by G. Hildebrandt ...]. - Frankfurt am Main ; Bern ; New York ; Paris : Lang, 1987.
(Proceedings of the ... annual meeting of the European Society for Chronobiology ; 2)
ISBN 3-8204-9882-6

NE: Hildebrandt, Gunther [Hrsg.]; European Society for Chronobiology: Proceedings of the ...

ISBN 3-8204-9882-6

Druck und Bindung: Weihert-Druck GmbH, Darmstadt

Printed in Germany



CONTENTS

I. BASIC AND CELLULAR ASPECTS

b) Data Acquisition and Processing

IV. COORDINATION AND INTERACTIONS

CIRCADIAN VARIATION OF THE ATROPINE EFFECT ON THE RUNNING PERFORMANCE OF MICE TREATED WITH 1,2,2-TRIMETHYLPROPYLMETHYL-PHOSPHONOFLUORIDATE (SOMAN)

H. ARBOGAST, M. HALLEK, B. ARBOGAST & L. SZINICZ

Institut für Pharmakologie und Toxikologie der Akademie des Sanitäts- und Gesundheitswesens der Bundeswehr - BSW, Ingolstädter Landstr. 100, D-8046 Garching-Hochbrück, FRG

INTRODUCTION

The efficacy of atropine as antidote in an organophosphate poisoning has been frequently documented (BERRY et al, 1966; HELM, 1980). This compound has become an indispensable component of the therapy. In contrast, little is known about the influence of atropine on the non-lethal effects of organophosphates.

The setup of this experiment arouse out of the observation that the toxic effects of many drugs change with the time of day. This could be demonstrated for a large variety of drugs (REINBERG & HALBERG, 1971) including paraoxon, the active metabolite of parathion, organophosphate and a potent cholinesterase inhibitor (MAYERSBACH, 1974) and soman, a very potent and rapid-acting organophosphate "nerve agent" (ELSMORE, 1981).

In this study, the influence of atropine on the motor performance of mice treated with soman and the circadian variation of this effect were investigated.

METHODS

White male NMRI-mice, weighing between 20 and 25 g with tap water and standard mice chow ad lib (Altromin) were used. The animals were allowed to adapt for 10 days and were then trained for three consecutive days, once daily, for 60 min. A mash wire drum (20 cm diameter) rotating at 14 rpm was used.

The animals received the compounds to be tested immediately before the running period of 60 min. Before the injection and after the experiment (running period) a modified neurologic behavioral screening program was performed, according to IRWIN (1968). 26 parameters were tested: body position, locomotor activity, exophthalmus, abnormal movements, tremor, convulsions (viewing glass); appearance, piloerection, eye opening, startle reaction (arena); visual placing, grip strength, corneal reflex, pinna response, toe and tail pinch responses, body tone and temperature (steel wire grid); positional struggle, abdominal and extremity tone, salivation, lacrimation, skin colour, diarrhea, respiratory rate, biting provocation (supine position). The differences between the single values before the injection and after the running period were formed

and summarized to five scores: activity, reflexes, muscle tonus, autonomous and abnormal behavior.

After randomization, 7 animals per group received either saline solution s.c. and i.p., or soman (50% LD_{50} s.c. and saline i.p., or soman (50% LD_{50} s.c. and atropine (10 mg/kg) i.p.

In a blind procedure, this setup was repeated at six different time points along the 24-h scale, with a total of 126 animals.

A detailed description of the method will be published elsewhere (ARBOGAST et al, in preparation).

STATISTICS

The results were checked for significant differences by oneway and twoway of variance (ANOVA, 1983) and rhythms were quantified by the cosinor method (NELSON et al, 1971; BINGHAM et al, 1982).

RESULTS

Primarily - independently from daytime - in animals receiving soman alone running performance, as well as the screening scores activity, reflexes, muscle tonus and autonomous behavior were significantly reduced, the abnormal behavior score was increased. Looking at the data in dependence of daytime (Fig. 1), for this group a significant circadian variation could be detected in the activity and abnormal behavior scores.

The animals treated with soman and atropine demonstrated a marked improvement in running performance, activity, reflexes, autonomous and abnormal behavior scores, compared to the animals receiving soman alone. Atropine treatment had no effect on muscle tonus, as expected.

A higly significant circadian variation, however, could be verified in running performance of this group (Fig. 1a), as well as in the screening scores activity and abnormal behavior (see also Table in Fig. 1). The clearly twin peaked data curves indicate the existence of an additional 12-h rhythm, which could be manifested by the cosinor method.

The most striking daytime variation, however, was prominent in the improvement of running performance by atropine therapy (Fig. 2a showing the %al improvement of running performance in the atropine therapy group compared to the respective animals receiving soman alone in dependence of daytime). Here, data vary between -63 and +223%.

If analyzed by the cosinor method (Fig. 2b), these data exhibit a significant circadian rhythm with an acrophase towards the end of the activity period of the mice (seen in circle A). Also, an ultradian 12-h rhythm could be manifested (shown in circle B).

Compared to the saline controls, an acrophase shift in the activity score of the groups receiving soman (with or

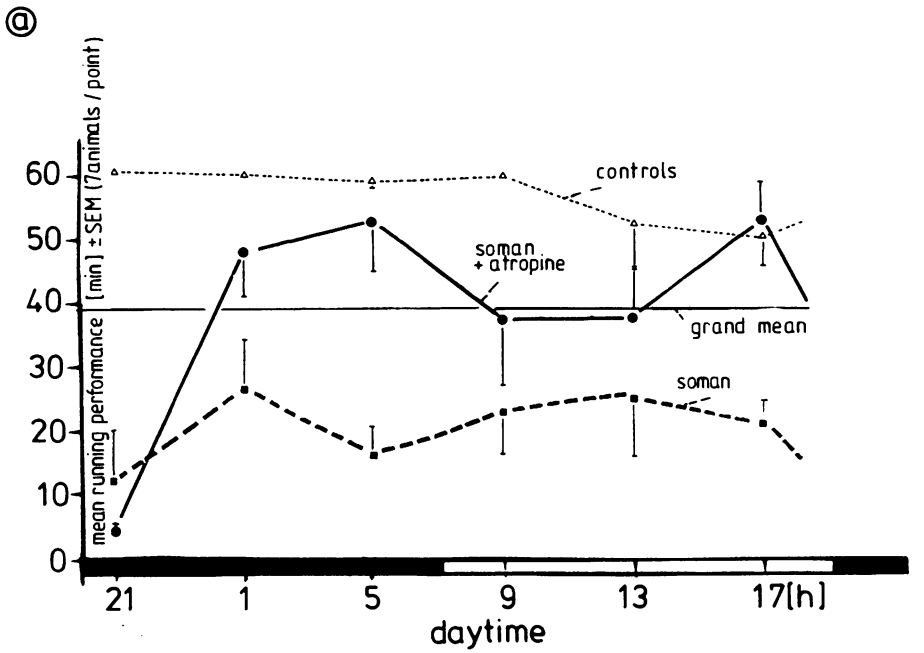
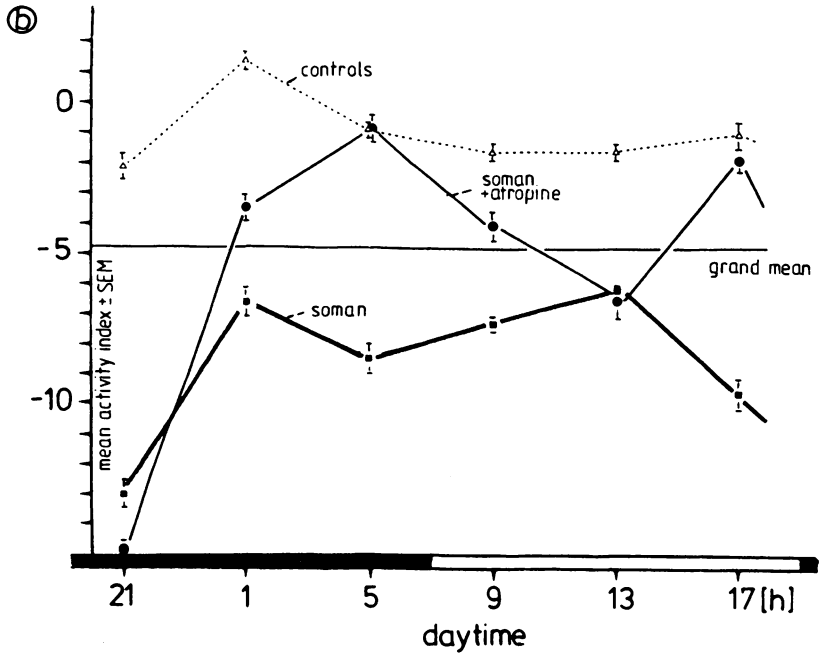
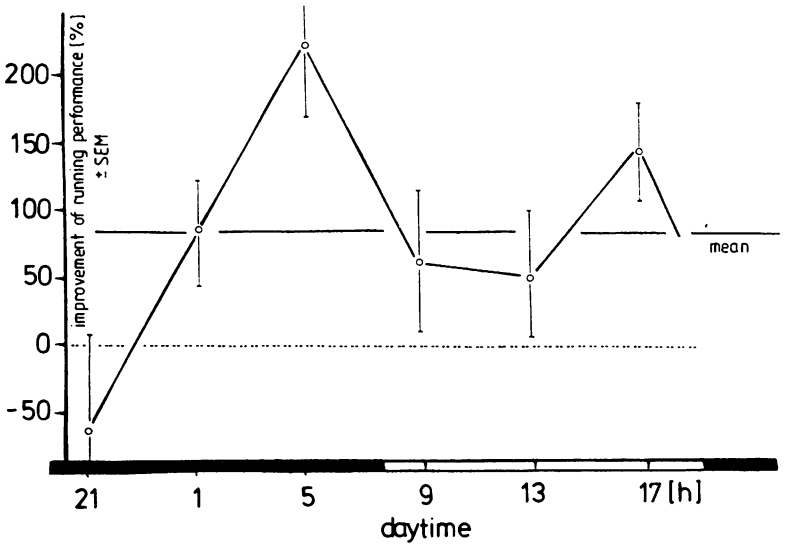


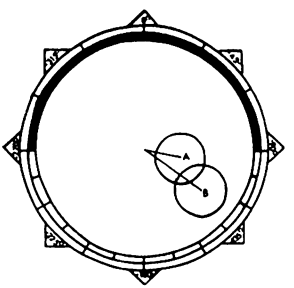
Fig. 1: a) Circadian variation of the mean running time (min.) in mice treated with saline, soman, soman+atropine;
b) Circadian variation of the mean activity score (arbitrary units) in mice treated with saline, soman, soman+atropine; each data point represents 7 animals; the inserted table demonstrates all time dependences obtained by ANOVA at different levels of significance.



		running performance	activity	autonomous behavior	abnormal behavior	reflexes	muscle tonus
one-way-analysis	CONTROLS		+	++	+		
	SOMAN		+		++		
	SOMAN+ ATROPINE	+++	++		++		
two-way-	THERAPY	+++	+++	+++	+++	+++	+++
	TIME	++			+++		

+ $p<0,05$
++ $p<0,01$
+++ $p<0,001$





SINGLE COSINOR

p	period [h]	n	mesor	amplitude ± SE	acrophase ± SE [h]
0.05	A: 24	42	83.9	58.6 ± 39.8	6.45 ± 2.40
	B: 12			108.2 ± 39.8	4.16 ± 0.41

Fig. 2: a) Circadian variation of the atropine effect on the mean running performance improvement (%) in mice treated with soman; n = 7 animals per time point; b) cosinor analysis of mean running performance improvement; the parameters of the 24- and 12-h rhythms are listed below the graph.

without atropine) could be observed. Activity acrophases in the latter groups showed a clear synchronicity with the acrophase of running performance improvement.

CONCLUSIONS

In conclusion it can be stated that the application of atropine clearly improved the effects caused by soman, except muscle tonus. The effect of atropine on motor behavior as well as on all the neurologic scores showed strong circadian variations, stronger than those in controls and in animals receiving soman alone. Acrophase synchronicity of running performance improvement with the rhythm of the activity score could also be shown. Hence, activity could thus be used as a "marker rhythm".

As one possible interpretation, it might be suggested that the rest-activity cycle of the animals also synchronizes the sensitivity of the organism towards atropine. The 12-h rhythms manifested in our activity data are also confirmed by literature, where 12-h rhythms in activity are commonly described (HALBERG & HALBERG, 1984).

ELSMORE (1981) reported about circadian variations in the toxic (lethal) effects of soman. His finding of a circadian rhythmicity in the susceptibility to "nerve agent" poisoning could be confirmed by our data for the non-lethal effects. In addition, the detection of a circadian dependence of the therapeutic atropine effect very well coincides with ELSMORE's findings demonstrating a significantly higher LD_{50} and a significantly lower body weight loss during the dark period. His cholinesterase measurements in three brain regions (brainstem, hypothalamus and midbrain) all exhibited peak concentrations during the dark span, when soman was least toxic.

We also found atropine efficacy highest during the dark period. So it might be suggested that as one of the possible mechanisms the circadian rhythmicity of brain acetylcholinesterase may cause the circadian dependence of the therapeutic atropine effect.

As a final remark, the data presented here show the necessity of considering the time of day when performing organophosphate toxicity experiments.

REFERENCES

1. ARBOGAST, H., HALLEK, M. & SZINICZ, L. (1987): Assessment of the motor performance and the neurologic state of the mouse: An alternative to LD experiments. In preparation.
2. BERRY, W.K., DAVIES, D.R. & RUTLAND, J.P. (1966): Problems in the treatment with oximes and atropine of rats poisoned by organophosphates. Biochem. Pharmacol. 15, 1529
3. BINGHAM, C., ARBOGAST, B., CORNELISSEN, G. & HALBERG, F.

(1982): Inferential statistical methods for estimating and comparing cosinor parameters. Chronobiologia 9, 397-412

4. ELSMORE, T. (1981): Circadian susceptibility to soman poisoning. Fundam. Appl. Toxicol. 1, 238-241
5. GROFF, W.A., KAMINSKIS, A. & ELLIN, R.I. (1976): Interconversion of cholinesterase activity units by the manual delta pH method and a recommended automated method. Clin. Toxicol. 9, 353-358
6. HALBERG, F. & HALBERG, E. (1984): Chronopharmacology and further steps toward chronotherapy. Pharmacokinetic Basis for Drug Treatment, pp. 221-248
7. HELM, U. (1980): Nervenkrampfstoffvergiftung (Alkylphosphatvergiftung). In: Wehrmedizin, E. Rebentisch (ed.). Urban & Schwarzenberg, München, pp. 247-260
8. IRWIN, S. (1968): Comprehensive observational assessment: Ia. A systematic, quantitative procedure for assessing the behavioral and physiologic state of the mouse. Psychopharmacol. (Berlin) 13, 222-257
9. MAYERSBACH, H. von (1974): Circadian liver detoxication and acetylcholinesterase rhythmicity: two limiting factors in circadian E600 toxicity. In: L.E. Scheving, F. Halberg & J.E. Pauly (eds.). Chronobiology, Igaku Shoin Ltd., Tokyo, pp. 191-196
10. NELSON, W., TONG, Y.L., LEE, J.-K. & HALBERG, F. (1979): Methods for cosinor rhythmometry. Chronobiologia 6, 305
11. REINBERG, A. & HALBERG, F. (1971): Circadian chronopharmacology. Annu. Rev. Pharmacol. 11, 455-492
12. Die Statistikprozeduren (1983). In: SPSS 9 - Statistik-Programm-System für die Sozialwissenschaften, 4. Aufl., Fischer-Verlag, Stuttgart-New York, pp. 105-266

AUTHOR INDEX