THE INFLUENCE OF ISOLATED SOMAN-INDUCED ERYTHROCYTE ACETYLCHOLINESTERASE INHIBITION ON BASIC PHYSIOLOGICAL FUNCTIONS IN RABBITS

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Summary

The influence of isolated soman-induced inhibition of erythrocyte acetylcholinesterase on the basic physiological functions (respiration and circulation) was evaluated in a rabbit model with on-line monitoring of respiratory and circulatory parameters. Following 24h incubation of rabbit erythrocytes with soman (10^{-3} M) in vitro, the erythrocytes were added into plasma and injected to intact anaesthetized rabbit. The respiratory and circulatory parameters were monitored till 120 min following the end of blood-transfusion. At the end of experiments, the blood and various organs were removed to measure cholinesterase activities.

No changes in monitored respiratory and circulatory parameters as well as no significant changes in cholinesterase activities with the exception of erythrocyte acetylcholinesterase were observed in comparison with controls. These data suggest that isolated significant decrease in erythrocyte acetylcholinesterase has not any influence on the basic physiological functions in rabbits.

KEY WORDS: Soman; Acetylcholinesterase; Transfusion; Respiration; Circulation; Rabbit.

Introduction

The enzyme acetylcholinesterase (AChE, EC 3.1.1.7) is responsible for terminating the transmitter action of acetylcholine (ACh) at the junction of the various cholinergic nerve endings with their effector organs or postsynaptic sites (1). The inhibition of AChE leads to an accumulation of ACh at cholinergic receptor sites and thus to production of effects equivalent to continuous stimulation of cholinergic fibers throughout the central and peripheral nervous systems (2).

Nevertheless, the AChE activity was also found in erythrocytes. Till now, the importance of erythrocyte AChE has not made clear. The erythrocyte AChE seems to hydrolyze ACh in the spleen and thus cause the haemolysis of the old erythrocytes by pH fall (3). It was previously shown that physostigmine reduced the destruction of erythrocytes in the spleen by its inhibiting effect on AChE activity (4).

The aim of this study was to evaluate the importance of erythrocyte AChE for basic physiological functions (respiration and circulation) by creating an animal model with isolated inhibition of erythrocyte AChE. The influence of isolated decrease in erythrocyte AChE on respiration and circulation was observed in a rabbit model with on-line monitoring of respiratory and circulatory parameters.

Material and Methods

Male rabbits (3.2-3.5 kg) obtained from Konárovice were kept in an air-conditioned room (20-22 °C) with light from 07.00 to 19.00 hrs and was allowed a free access to standard laboratory food and tap water. Handling of experimental animals was made under the supervision of the Ethics Committee of the Medical Faculty of Charles University and the Military Medical Academy in Hradec Králové. After exsanquination of the anaesthetized rabbit (urethan i.p., 1g/kg b.w.), the blood was centrifuged to obtain erythrocytes and plasma. Erythrocytes were incubated for 24h with a highly toxic organophosphorus agent soman (10^{-3} M) to completely inhibit AChE activity in erythrocytes (5). Following the incubation and separation of erythrocytes from soman solution by centrifugation, erythrocytes were

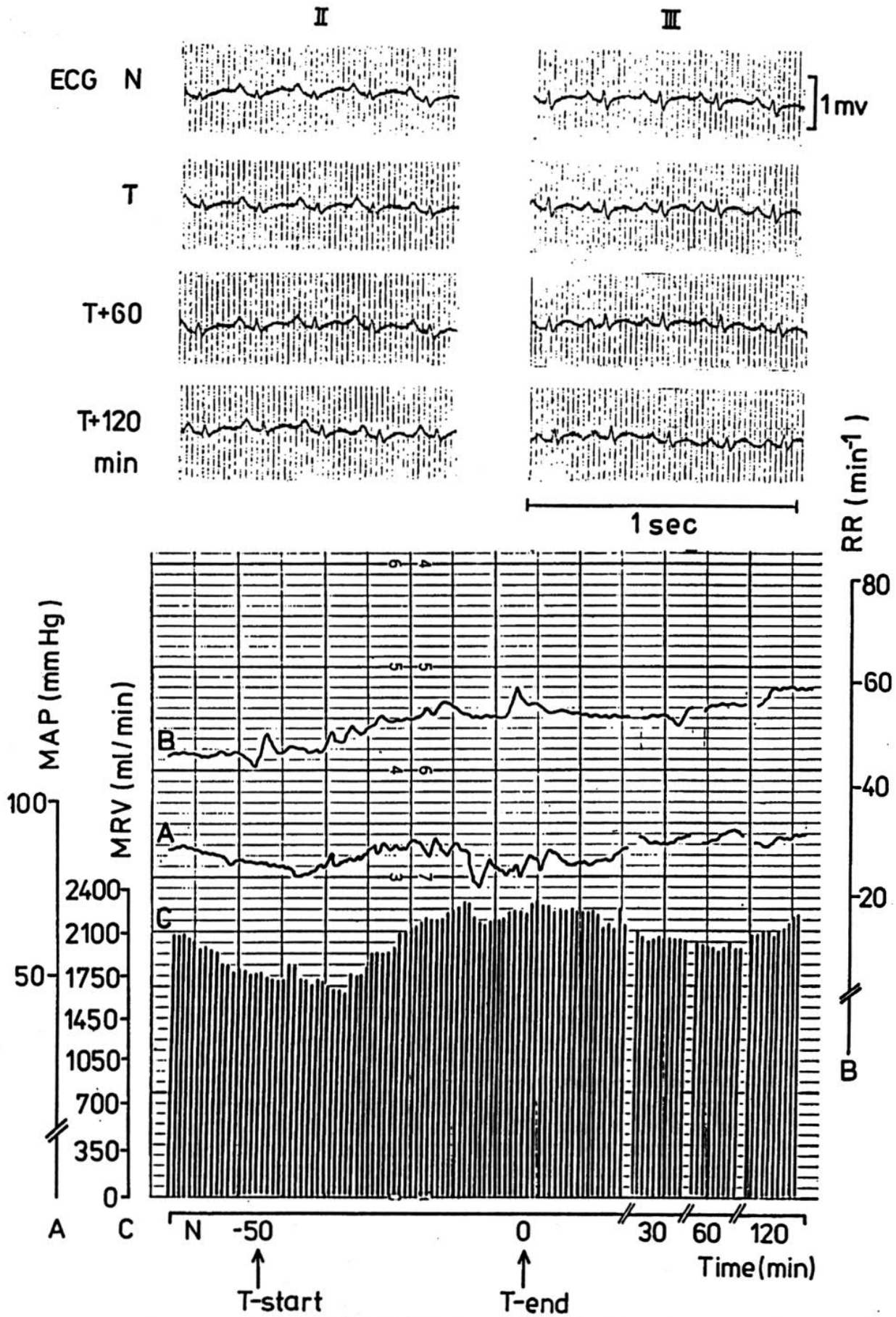

Fig. 1 Copy of graphic registration of actual values of respiratory rate (RR), minute respiratory volume (MRV), mean arterial pressure (MAP) and electrocardiogram (ECG) in control rabbit where N - before transfusion with the blood containing intact erythrocytes, T-start - the onset of transfusion, T-end - the end of transfusion.

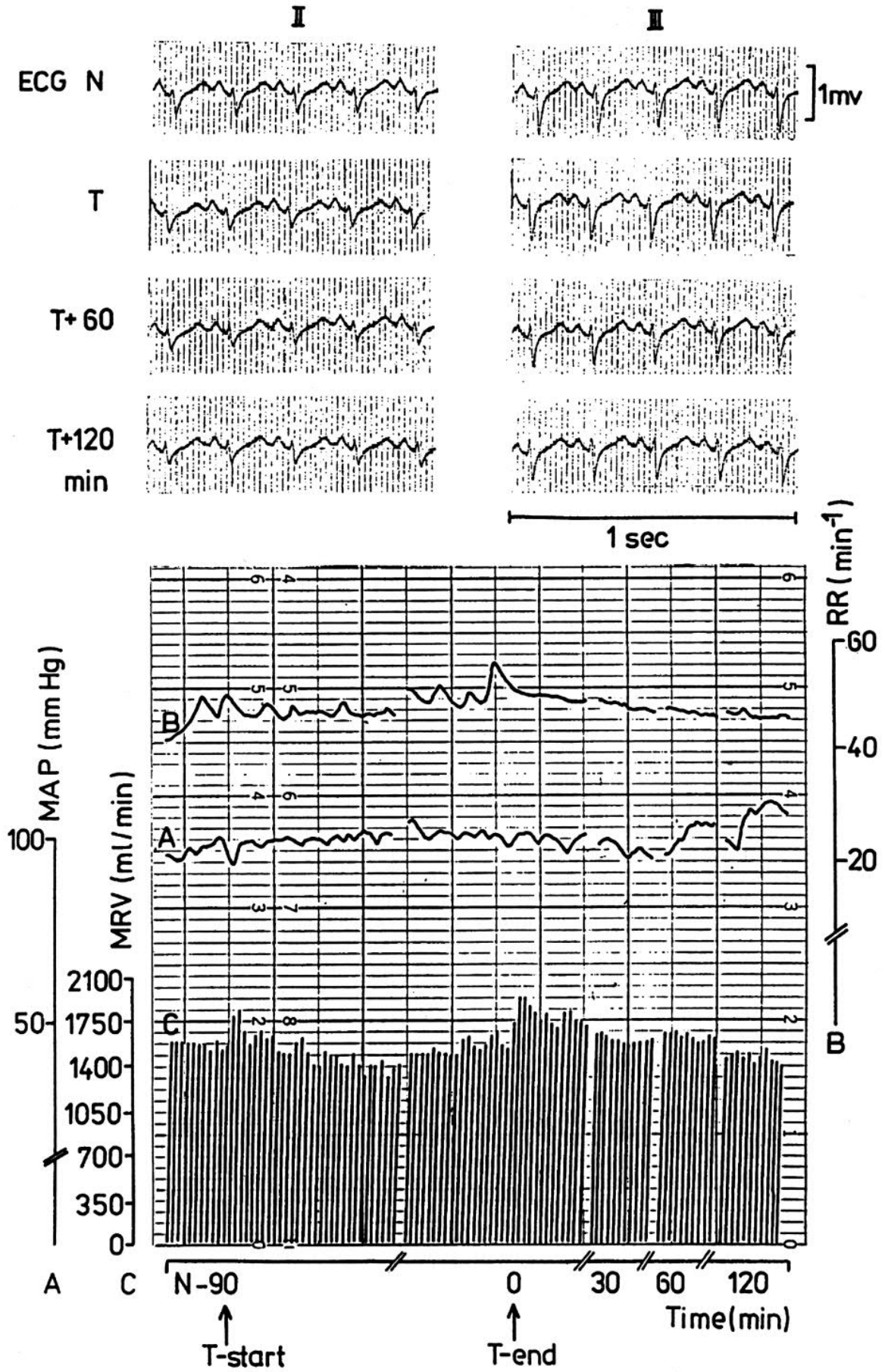

Fig. 2 Copy of graphic registration of actual values of respiratory rate (RR), minute respiratory volume (MRV), mean arterial pressure (MAP) and electrocardiogram (ECG) in experimental rabbit where N - before transfusion with the blood containing soman-treated erythrocytes, T-start - the onset of transfusion, T-end- the end of transfusion.

washed with saline solution three times to remove free soman (6).

The erythrocytes were added to rabbit plasma and the exchanged blood-transfusion (30 ml/kg b.w.) was performed to another rabbit that had been anaesthetized and heparinized (heparin i.v., 850 Units/kg b.w.) to prevent coagulation before transfusion. Then the rabbit was positioned on a heatable operating table, the trachea and the left carotid artery were prepared after medical incision of the throat. A tracheal cannula was inserted and connected to a pneumotachygraph according to Fleisch (Hugh Sachs, Electronics, Germany). The carotid artery was cannulated with teflon catheters and connected to a pressure transducer (Tesla LMP 102, Valašské Meziříčí, Czech Republic). Respiratory rate, minute respiratory volume, heart rate and mean artery pressure were on-line recorded by a multiple-pen recorder (Rikadenski Electronics, Germany). Electrocardiogram was registered using subcutaneous needles and EGG NEK 401 instrument (Messgärette-Zwönitz, Germany).

After a stabilization period of 10-15 min, the baseline values were recorded for 5 min. Then, the exchanged blood-transfusion was provided. The number of animals was five in the control (blood-transfusion with erythrocytes without incubation with soman) as well as experimental group. The experiments were terminated 120 min following the end of the transfusion. The respiratory and circulatory data were recorded up to this point. At the end of experiments, the rabits were killed by air embolism into the carotid artery and the blood, diaphragm, liver and brain were removed. The brains were dissected in various parts and hemispheres, cerebellum, medulla oblongata and pons Varoli were chosen for the measurement of AChE activity. The blood was centrifuged to obtain erythrocytes and plasma. The erythrocytes were haemolysed and the diaphragm, liver and brain parts were homogenized in distilled water. The AChE and butyrylcholinesterase (BuChE, EC 3.1.1.8) activities were measured by a spectrophotometric assay using acetylthiocholine or butyrylthiocholine as substrates (7).

The total AChE and BuChE activities were expressed as μmol substrate hydrolyzed/ml(mg)/min and presented as percents of controls. Statistical significance was determined by the use of Student's t-test and differences were considered significant when $p < 0.05$. Statistical evaluation was performed with relevant computer programs (8).

Results

The absolute baseline values of respiratory and circulatory parameters were between 54.7 ± 6.2 and 58.7 ± 5.7 min^{-1} (respiratory rate), 1424.1 ± 106.0 and 1506.5 ± 121.3 ml min^{-1} (minute respiratory volume), 292.3 ± 5.9 and 301.6 ± 7.8 min^{-1} (heart rate) and 78.9 ± 8.2 and 90.8 ± 9.6 mm Hg (mean artery pressure). There were no significant differences between the control and experimental groups at baseline. The influence of isolated soman-induced inhibition of erythrocyte AChE on respiration and circulation is demonstrated in Figs. 1-2. The values of respiratory as well as circulatory parameters, recorded during the exchanged blood-transfusion and following the transfusion, are practically the same in both, control and experimental groups. These results suggest that the substitution of intact erythrocytes by erythrocytes without AChE activity has not any influence on respiration and circulation in experimental animals.

The values of AChE and BuChE activity in the erythrocytes, plasma, diaphragm, liver and various parts of the brain 120 min following the end of blood transfusion with intact or soman-treated erythrocytes are shown in Fig. 3. The erythrocyte AChE is significantly decreased to 48 % of control values ($p < 0.05$) because the erythrocytes without AChE activity were injected into experimental rabbits. On contrary, the other AChE or BuChE activities are not significantly changed in comparison with controls.

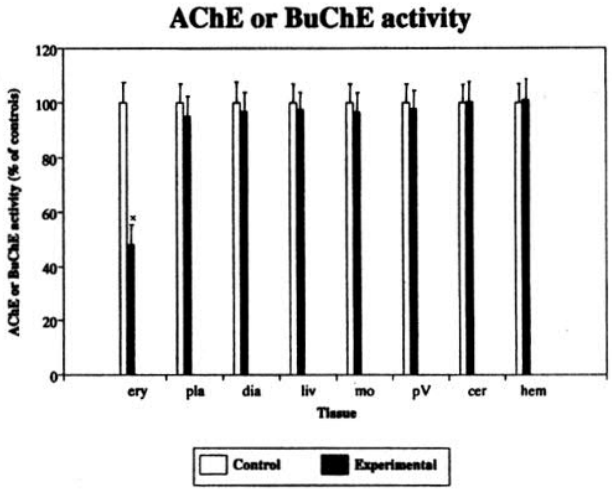

Fig. 3 Changes in rabbit AChE activity in the erythrocytes (ery), diaphragm (dia) and various parts of the brain (hemisphere - hem, cerebellum - cer, pons Varoli - pV, medulla oblongata - mo) and in rabbit BuChE activity in plasma (pla) and liver (liv) following the transfusion with the blood containing soman-treated erythrocytes. Statistical significance between two compared values - ^{x} $p < 0.05$.

Discussion

The erythrocytes without AChE activity were prepared in vitro by 24 h incubation of rabbit erythrocytes with high concentration of soman to achieve zero AChE activity without spontaneous

reactivation. It was found that soman is able to release from erythrocytes after binding on them (6). Nevertheless, the rate of soman releasing depends on the time of its incubation with erythrocytes. Following 24 hrs incubation soman released from erythrocytes in a concentration unable to significantly inhibit tissue cholinesterase activities (9). That is why only erythrocyte AChE activity was significantly decreased following the exchanged blood-transfusion with soman-treated erythrocytes in comparison with controls.

The values of respiratory as well as circulatory parameters in rabbits with isolated significant decrease in erythrocyte AChE activity did not differ from the values of respiratory and circulatory parameters in control rabbits. These data confirm that erythrocyte AChE seems not to play any role in respiration and circulation in rabbits.

In conclusion, the erythrocyte AChE activity serves as a valuable marker of exposure to irreversible and reversible AChE inhibitors (2) but its full activity is not necessary for survival of experimental amimals.

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