On the action of vitamin C on the neuromuscular junctions

C.I. Triantaphyllidis, V.P. Papageorgiou[1] and G. Logaras

Department of Experimental Pharmacology, Medical Faculty, Aristotle University of Thessaloniki, Saloniki (Greece), 26 September 1978

Summary. High concentrations of ascorbic acid produce complete blockage of the transmission of nervous stimuli on the isolated neuromuscular preparation of phrenic nerve-hemidiaphragm of the rat.

During the current decade, especially after the publications of Pauling[2,3] it is widely recommended to administer high doses of ascorbic acid not only for treatment but also for prevention in a number of diseases. It was therefore decided to investigate the activity of high concentrations of ascorbic acid in the transmission of the nervous stimuli on the neuromuscular synapse.

The method was based on that of Bülbring[4]. The phrenic nerve was stimulated with impulses from a square-wave stimulator. Supramaximal shocks of 4 V, 500 μsec at a frequency of $^1/_{15}$ sec were applied. Drugs used were: L (+) ascorbic acid, neostigmine methylsulfate, d-tubocurarine chloride and succinylcholine bromide.

The administration of neostigmine, in concentration 2.9 M, as soon as ascorbic acid in concentration smaller (0.017 M) than that producing complete neuromuscular block (0.034 M), was added to the bath and had as result the inhibition of neuromuscular transmission on the preparation, and complete neuromuscular blockage (figure 2, a). The administration of d-tubocurarine in concentration of 2.45 M, 5 min after the effect of 0.025 M concentration of ascorbic acid on the same preparation, resulted in the inhibition of the effect of d-tubocurarine in a sufficient grade (figure 2, b). Finally, the simultaneous administration of ascorbic acid and succinylcholine, in concentrations

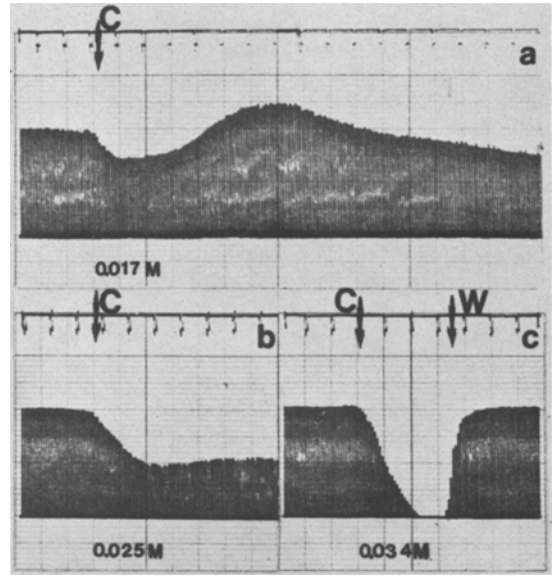


Fig. 1. Action of ascorbic acid on the isolated neuromuscular preparation of phrenic nerve-hemidiaphragm of the rat.

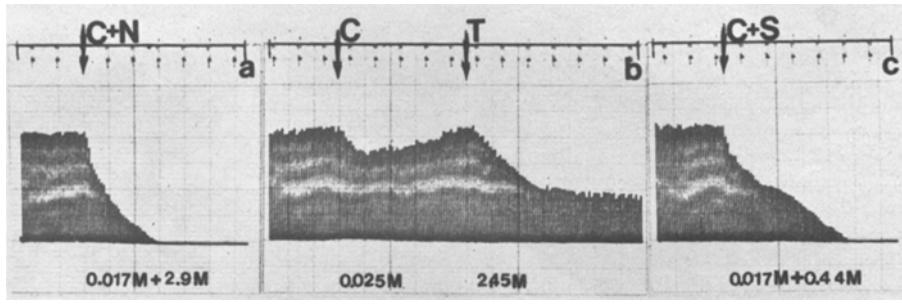


Fig. 2. Action of the simultaneous administration of ascorbic acid-neostigmine (a), ascorbic acid-d-tubocurarine (b) and ascorbic acid-succinylcholine (c).

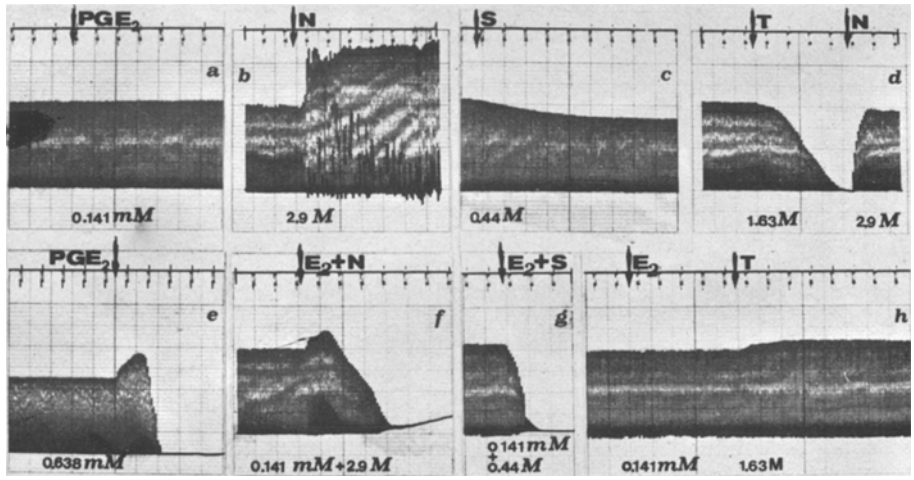


Fig. 3. Action of PGE_2 (a, e), neostigmine (b), succinylcholine (c), d-tubocurarine (d) and of the combination PGE_2-neostigmine (f), PGE_2-succinylcholine (g), PGE_2-d-tubocurarine (h) on the isolated neuromuscular preparation of phrenic nerve-hemidiaphragm of the rat.

which, when given separately, do not produce complete neuromuscular blockage (figures 1,b and 3,c), rapidly provoked complete block of the transmission of nervous stimuli (figure 2,c).

It has been published[5] that high concentrations of ascorbic acid block the transmission of nervous impulses on the isolated neuromuscular preparation of phrenic nerve-hemidiaphragm of the rat, and that this kind of block was not reversed by neostigmine or calcium.

Our results indicate that ascorbic acid interacts with muscle relaxants and neostigmine, and that the block produced has the same characters as that which is produced by prostaglandins[6,7] (figure 3). The prostaglandins A_1, A_2, E_1, E_2 and B_1 block the transmission of nervous impulses on the isolated neuromuscular preparation of phrenic nerve-hemidiaphragm of the rat. This effect has an indirect mode of action, which is due to the liberation of big amounts of the transmitter by ascorbic acid (figure 1,c). According to these observations, it is possible to explain the results produced by the simultaneous administration of ascorbic acid, in concentrations smaller than that producing complete neuromuscular blockage, and neostigmine (figure 2,a). On the other hand, antagonism was found between ascorbic acid and d-tubocurarine, to the production of neuromuscular blockage (figure 2,b). This is because the amount of acetylcholine released by the nervous stimuli in the presence of ascorbic acid is large, and competes with the d-tubocurarine, producing a biological competition. Finally, the synergism found between ascorbic acid and succinylcholine (figure 2,c), suggests that these substances are working actively producing neuromuscular blockage by the same mechanism.

1 Laboratory of Organic Chemistry, College of Engineering, Department of Chemical Engineering, Aristotle University of Thessaloniki, Greece.

2 L. Pauling, Proc. natl Acad. Sci. USA, *67*, 1643 (1970).
3 L. Pauling, in: Vitamin C and the Common Cold. W.H. Freeman Co., San Francisco 1970.
4 E. Bülbring, Br. J. Pharmac. *1*, 38 (1946).
5 V.P. Papageorgiou, C.I. Triantaphyllidis, V. Mirtsou-Fidani, A. Mellides and G. Logaras, in: Annual of the Faculty of Medicine, vol. 11, p. 131. University of Thessaloniki 1975.
6 C.I. Triantaphyllidis, Thesis, University of Thessaloniki 1978.
7 C.I. Triantaphyllidis and G. Logaras, in: Annual of the Faculty of Medicine, vol. 12, p. 95, University of Thessaloniki 1978.

Diurnal rhythm of ethanol metabolism in the rat[1]

K.F.A. Soliman and C.A. Walker

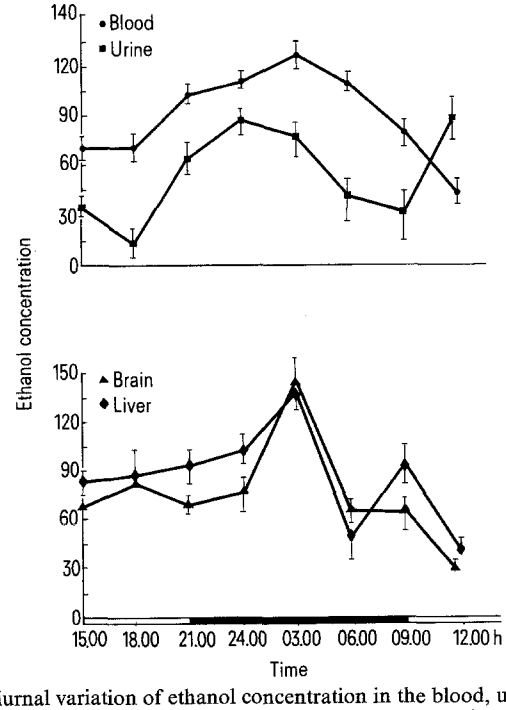
School of Pharmacy, Florida A & M University, Tallahassee (Florida 32307, USA), 25 September 1978

Summary. Male Sprague-Dawley rats injected with 2.0 g/kg of ethanol and analyzed 1 h later at 8 specific times of the day showed diurnal rhythms for alcohol concentrations in the blood, urine, brain and liver tissues. The circadian fluctuation noted for the concentrations of blood and tissue ethanol might indicate a diurnal variation in the enzymatic metabolism of ethanol.

Many studies have demonstrated that the effect of a drug, chemical or poison has, is circadian-phase dependent. Ethanol toxicity was found to be circadian-phase dependent[2,3]. The circadian variations in drug response can be attributed to several factors including mechanisms associated with changes in target enzymes, variation in the drug absorption rate and fluctuation in the rate of excretion. In an attempt to exploit the circadian behavior of ethanol effect this work was undertaking to measure the variation of ethanol levels in rats treated with a fixed ethanol dose.

Methods. Male Sprague-Dawley rats weighing approximately 150 g each were used in these experiments. The animals were adapted to a 12 h light/12 h dark cycle for a minimum period of 3 weeks prior to treatment. The temperature was maintained at 23±1 °C, and the animals were fed and watered ad libitum. The test animals were injected with 2.0 g/kg of ethanol at 8 different times of the day, 1 h prior to sacrificing. Immediately after sacrificing, the blood was collected in heparinized tubes and was centrifuged. Brain and liver samples were dissected out and stored at −20 °C until analyzed. The brain and liver portions were homogenized in distilled water equivalent to 8 times the tissue weight. Deproteinization of tissue samples was accomplished by 6.25% trichloroacetic acid. Ethanol levels were determined using an enzymatic procedure[4].

The analysis of data obtained was based on the establishment of a regression line for standards and samples. The readings were then converted to mg% ethanol using the regression equation from the standard line. The regression equation used ($\hat{y} = -21.1 + 204.3x$) has a 0.98 coefficient of determination. The data was further subjected to analysis



Diurnal variation of ethanol concentration in the blood, urine, liver and brain. Each point represent the average of 8 animals ±SEM. The shaded area of the graph represents the dark phase. Ethanol concentration is expressed as mg/100 ml of body fluids or as mg/100 g of tissue studied.