Effects of haloperidol, methylergometrine and phentolamine on the frog ERG

P. Kupenova and S. Belcheva

Department of Physiology, Medico-Biological Institute, Medical Academy, Sofia 1431 (Bulgaria), 20 November 1980

Summary. The effects of $3 \cdot 10^{-5}$ M solutions of haloperidol (HAL), methylergometrine (ME) and phentolamine (PHA) in a dose of 27 µl on the ERG of isolated frog eye cup preparations were examined. HAL induced a decrease in the amplitude of both the b- and d-waves which began about 20 min after the application. The dominating ME effect was a fast increase of the b-wave amplitude, while PHA did not induce any significant influences on the ERG.

According to biochemical data[1-3] dopamine (DA) is the dominating catecholamine in the retina of vertebrates. DA-containing neurons belong mainly to the amacrine cells (ACs), and in some species to the interplexiform ones[4-8]. Less is known about the role of the DA-ergic neurons in retinal functions. Exogenous DA causes on the one hand an inhibition of the ganglion cells' firing rate[9,10] and on the other, a depolarization of some types of horizontal cells[4,11,12] resulting in an attenuation of the lateral inhibitory influences in the outer plexiform layer[4].

In the present study we examined the effects of some drugs known to act as DA antagonists as haloperidol (HAL), methylergometrine (ME) and phentolamine (PHA)[4,11-18] on the frog ERG in order to obtain some insight into the possible role of the endogenous DA in the distal retinal layers. The frog retina with DA-ergic neurons found almost exclusively among the ACs[6] is a suitable object for this purpose because of its high DA content[3].

Materials and methods. The experiments were carried out on isolated eye cup preparations from a frog *(Rana ridibunda)* kept at a constant temperature (16 °C) with a continuous supply of moistened oxygen. Intermittent diffuse white light stimulation was used with light and dark periods of 5 sec. Illumination at the retinal level was 36 lx. The ERG was recorded with nonpolarized Ag/AgCl electrodes at a time constant of 1 sec.

HAL (Richter, Hungary), ME (VEB Arzneimittelwerk, GDR) and PHA (Merck, FRG) were dissolved in a modified bicarbonate Ringer's solution, containing 2% vitreous humour, in a concentration of $3 \cdot 10^{-5}$ M. Lactic acid was added to the HAL and corresponding control Ringer's solution in a concentration of 10 mg% as a stabilizer. A particular test or control solution (pH 8.2–8.3) was applied directly into the eye cup by means of a cannula in a dose of 27 µl, the pH final value and the effective drug concentration being lower due to the dilution by the residual fluid in

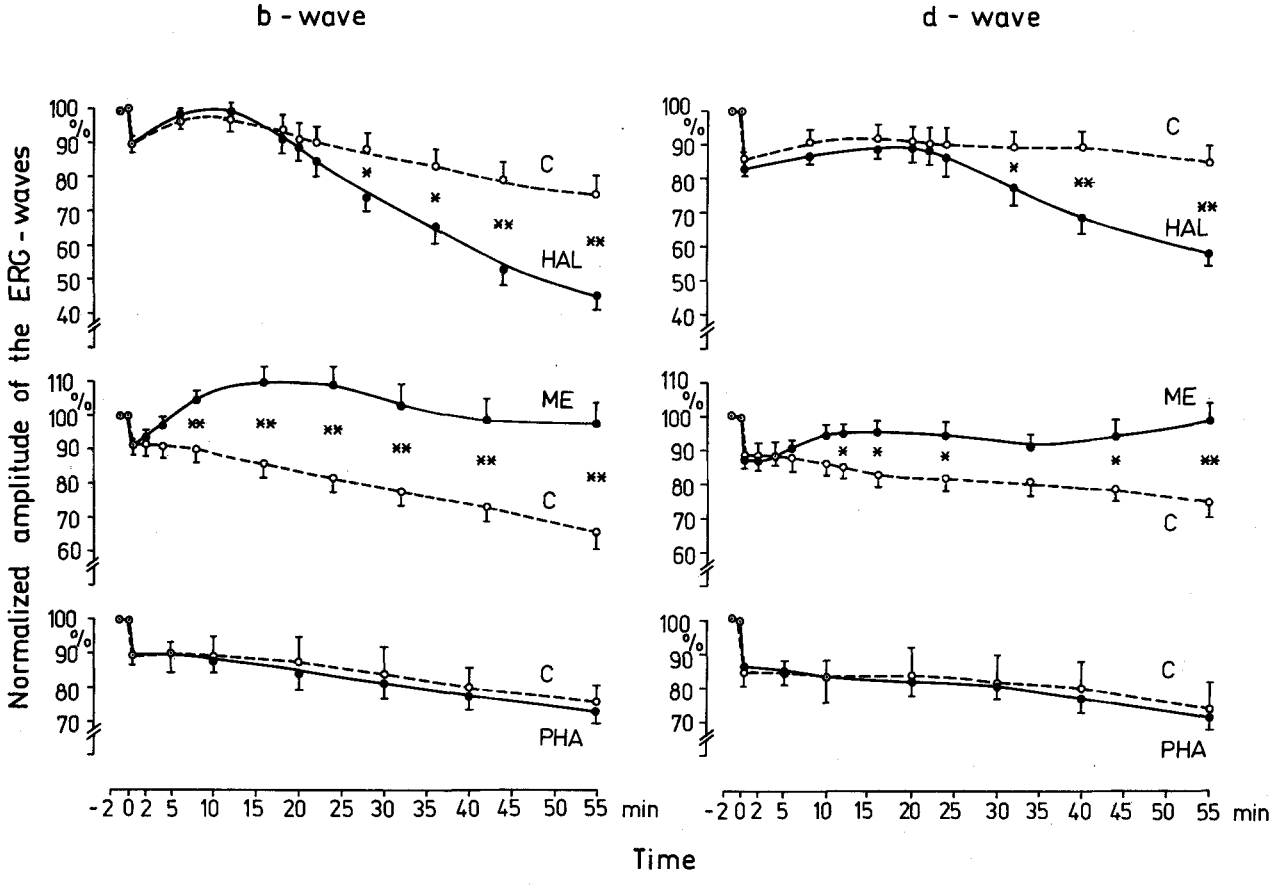

Figure 1. B-wave and d-wave amplitude changes in the ERG of frog eye cup preparations after haloperidol (HAL), methylergometrine (ME) and phentolamine (PHA) normalized to 100 at zero time, the moment immediately before the drug application. The control curves are denoted with C. Points on the curves represent mean values from 10 experiments. Vertical bars show SEM. Asterisks indicate significant differences from control measurements (* $p < 0.05$; ** $p < 0.01$; Student's t-test).

the eye cup. The application was performed after 2-3 min of light stimulation when the ERG became sufficiently stable (in the course of 10 subsequent recordings on the average). The changes in the amplitude (measured from peak to peak) and the shape of the ERG waves were followed for about 60 min after treatment. By comparison with the superfusion technique our method of application provided better conditions to measure the latency, but yielded less information on the reversibility of the drug effects.

Results. The results obtained with HAL, ME and PHA are presented in figure 1. After HAL a decrease of both b- and d-wave amplitude was observed. These changes began about 20 min after HAL application and developed progressively till the end of the experiments. The b- and d-waves in the corresponding control ERG were more stable after a small diminution (caused by the shunting effect of the applied solution) and the subsequent partial recovery. By contrast, ME induced an increase of the b-wave amplitude which became evident within the first min of its application and was accompanied by a shortening of the b-wave rise time (fig. 2). A less pronounced increase of the d-wave amplitude was also observed. The ME effects attained their maximum 15-20 min after the application and remained practically the same until the end of the experiments. There was a tendency for an additional increase of the d-wave amplitude 45-50 min after ME application. HAL and ME did not induce any significant changes in the a-wave amplitude nor in the latency of the ERG waves ($p > 0.05$ vs controls). After PHA no significant effects on the ERG became apparent.

Discussion. Our results show that HAL and ME change in a specific way the frog ERG while the α-blocker PHA in an equal dose has practically no effect. This indicates that probably the catecholaminergic receptors in the distal retina of the frog eye differ from the α-adrenergic ones. In agreement with the data that HAL acts as a DA antagonist mainly on the DA_e (excitation-mediating) receptors while the ergot drugs of the ergonovine group affect the DA_i (inhibition-mediating) receptors[13-15] one may presume that the opposite effects of HAL and ME on ERG result from the blocking of distinct types of receptors. Thus the decrease of the ERG waves after HAL may be due to the blocking of some DA_e receptors, whereas the b-wave enhancement after ME may result from the blocking of some DA_i receptors. The different time course of the ME and HAL effects on ERG found in our experiments is of particular interest. As the time of ME and HAL access to

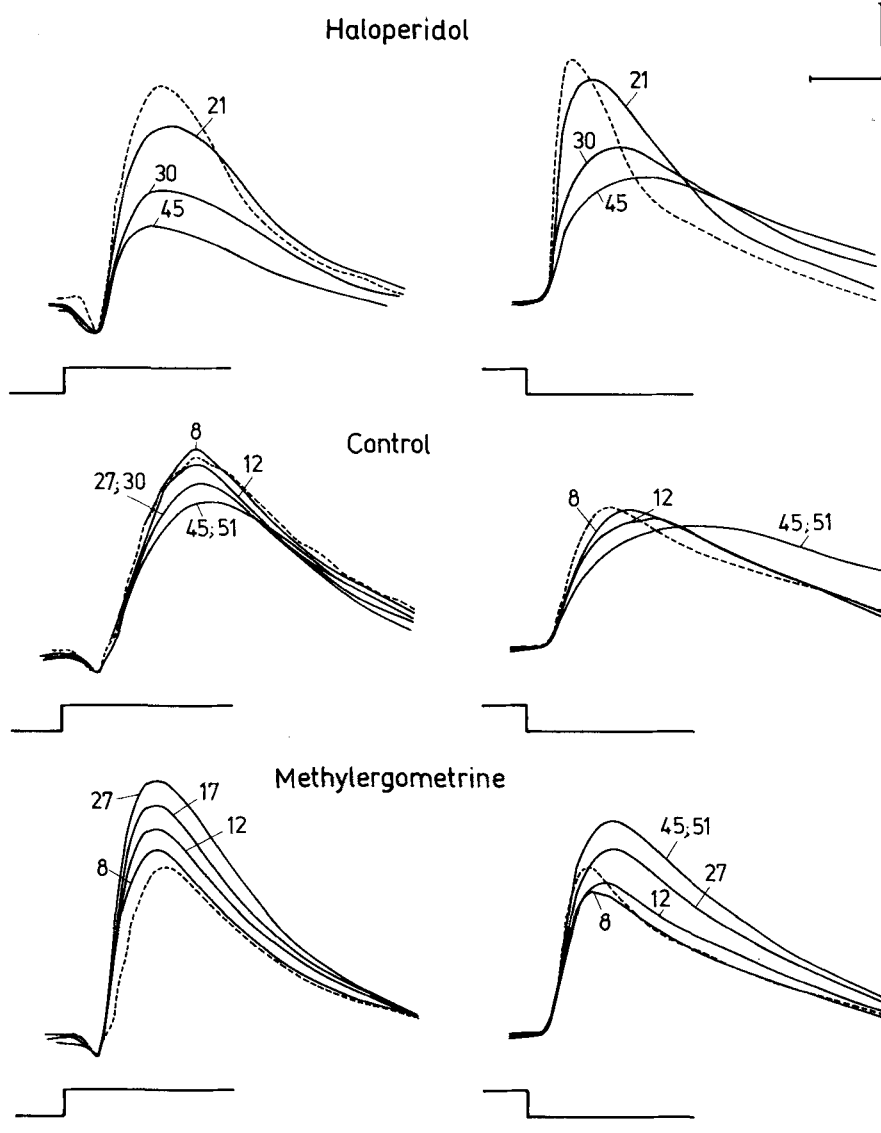

Figure 2. Effects of HAL and ME on the slope and amplitude of the ERG potentials of frog eye cup preparations, recorded at different time interval after drug application. The ERG curves photographed from the screen of an oscilloscope are magnified, superimposed and copied so that the beginnings of the b-waves (on the left) and d-waves (on the right) from a single experiment coincide. The ERG potentials before the application are represented by dashed lines; after the application by solid lines. The numbers indicate the time (min) after drug application. ERG curves from a control experiment are also presented. Calibrations: 250 µV, 200 msec. The marks for stimulus onset (_┌) and cessation (¬_) coincide only with the pretreatment potentials.

the neuronal network in the eye cup preparation is not expected to vary to such an extent, the possibility remains that the effects of retinal DA mediated by DA_i and DA_e receptors differ in their time course. The effects mediated by the DA_i receptors are thought to develop faster. A participation of the DA-ergic neurons in a negative feed back circuit between the on-type bipolar cells (BCs) and the ACs seems plausible. This is in agreement with the finding that exogenous DA induces a hyperpolarization of the on-type BCs[4]. Such a possibility is also compatible with some models[19,20] suggesting that the on-type BCs activity underlies the K^+ fluxes responsible for b-wave generation. In the case of HAL probably a more complex mechanism is involved. Recent data showing that exogenous DA penetrates the nuclei of the retinal neurons[21] indicate that retinal DA may participate in the control of long-term events such as RNA synthesis and protein metabolism. We presume that the delayed effects of HAL on ERG partially depend on its influencing these metabolic events. As HAL inhibits the DA effect on the DA-sensitive adenyl cyclase in retinal homogenates[16-18] one may assume that the effects of HAL on ERG may be mediated by its influence on some adenyl cyclase systems coupled to the DA_e receptors.

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Changes in the lipoproteins of rabbits on a high-fat, cholesterol-free diet; preventive action of metformin

C. Lacombe and M. Nibbelink

Institut de Physiologie, Université Paul Sabatier, ERA 412 CNRS, Rue F. Magendie, F-31400 Toulouse (France), 3 December 1980

Summary. Endogenous hypercholesterolemia induced by a cholesterol-free, high-fat diet corresponds to an increase in the level of low density lipoproteins and their enrichment in cholesterol esters. Metformin has no effect on the rise in plasma cholesterol but completely prevents the appearance of cholesterol-rich low-density lipoprotein.

It has been clearly established that cholesterol-induced atherosclerosis, in rabbits, is accompanied by characteristic changes in circulating very low density lipoprotein (VLDL) and low density lipoprotein (LDL)[1-4]. Lipoproteins modifications were also seen with other atherogenic diets[5-7].

In order to confirm the relationship between the lipoprotein composition changes and the higher incidence of atherosclerosis, investigations into the influence of other factors are of particular interest. The present work reports the influence of metformin (N,N-dimethylbiguanide) on lipoprotein changes induced by a high-fat, cholesterol-free diet. Metformin is a drug used in the treatment of diabetes which is well known for its preventive effects on cholesterol-induced atherosclerosis[8,9].

Techniques. Animals and diets. Fauve de Bourgogne male rabbits, weighing on average 2.5 kg at the start of the 3-month experiment, were divided into 3 groups of 10 and received the following treatments: chow diet, high-fat diet, high-fat diet and a daily dose of 120 mg/kg of metformin in 2 treatments per day. The composition of the high-fat diet used was: 10% coconut oil; 10% butter, 16% protein; 44% carbohydrate; 12.5% cellulose, 7.5% salt and vitamin mixture.

Lipid and lipoprotein analysis. Following an 18-h fast, the animals were killed and the blood collected over EDTA (1 mg/ml). Total plasma cholesterol[10] and triglycerides[11] were measured. Lipoprotein fractions: VLDL ($d < 1.006$), LDL ($1.019 < d < 1.063$), and high density lipoprotein (HDL) ($1.063 < d < 1.21$) were separated by ultracentrifugation on a KBr density gradient[12] in a Beckman SW 41 rotor. The composition of each class of lipoprotein was determined by measuring the levels of protein[13], triglycerides[11], total and free cholesterol[10], and phosphorus[14]. Phosphorus was converted to phospholipids by multiplying by 25. The amount of cholesteryl esters was calculated as the difference between total and free cholesterol and multiplied by 1.67. Quantitative analysis was carried out as previously described[15]. Plasma lipoprotein levels were calculated using the proportion of cholesterol obtained in the qualitative analysis. The liver cholesterol content[16] and total fatty acids[17] were determined after extraction with a chloroform-methanol (2:1, v/v) mixture.

Results. Fat-fed rabbits showed an increase in their plasma cholesterol while triglycerides were not significantly modified. Metformin had no effect on the hypercholesterolemia induced by the fatty diet and it increased plasma triglycerides. Lipid overload of the liver was also observed with the fatty diet. Metformin completely prevented accumulation of cholesterol in the liver (table 1).

Results concerning the lipoprotein pattern are presented in