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Effects of phosvitin on the ecg changes induced under hypoxia in the rat

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The effect of phosvitin (1 g kg^{-1}, i.p.) on ecg changes induced in rats by a reduction of partial oxygen pressure in the respiratory mixture was studied. Phosphocreatine, phosphoserine, ATP and $Na_2HPO_4.2H_2O$ were also administered intraperitoneally for comparison. Phosvitin alone was found to prevent the hypoxia-induced T-wave changes (flattening or disappearance), which were also temporarily aggravated by injection of noradrenaline. As to the metabolic, hypoxia-induced myocardial changes, two hypotheses are discussed: a release of phosvitin phosphate radicals ready for immediate utilization or a drug action mediated via a membrane-bound intrinsic proteinkinase system.

Phosvitin is the principal phosphoprotein of egg yolk (Mecham & Olcott, 1949). Isolated from egg yolk by many authors (see Wallace, Jared & Eisen, 1966 for references), it was also chemically characterized (Connelly & Taborsky, 1961; Allerton & Perlmann, 1965; Ramachandran & Sampath Kumar, 1967; Grizzuti & Perlmann, 1970). It has been hypothesized that the phosphoprotein (mol. wt $\simeq$ 40 000, Taborsky & Mok, 1967; phosphorus content 10%, Rabinowitz & Lipmann, 1960) acts as a supplier of energy-rich phosphate (Rabinowitz & Lipmann, 1960) or as an iron carrier (Greengard, Sentenac & Mendelsohn, 1964).

In earlier experiments (Caprino, Borrelli & Falchetti, 1973) we had shown that phosvitin has a protective effect on vasopressin-induced ecg changes, particularly on T-waves. These changes may result (Fresia, Mortari & others, 1964) from an ischaemic myocardium following a reduction in coronary flow.

Based on these findings, the present study was conducted in rats to test phosvitin's effects on the ecg pattern during induced hypoxia. In fact, it is known that experimental hypoxia produces certain characteristic ecg changes such as S-T segment depression, inversion of T-wave and premature ventricular beats (Sayen, Sheldon & others, 1958; Scheuer & Brachfeld, 1966; Pool, 1970; De Wall, Vasko & others, 1971).

MATERIALS AND METHODS

Procedure

Male Wistar rats (300 g) under sodium pentobarbitone anaesthesia (35 mg kg^{-1}, i.p.) were subjected to the following experimental design:

Phase I. Rats breathed atmospheric air for 30 min; no treatment was instituted.

Phase II. Rats breathed for 15 min, via the cannulated trachea, a mixture of nitrogen (70%) + atmospheric air (30%), delivered through a gas-mixing apparatus consisting of two electrically controlled flowmeters connected to a Palmer respiratory pump. Following 10 min of hypoxia, 3 μg kg^{-1} of noradrenaline was administered into the right jugular vein. Five min later, hypoxia was interrupted.

Phase III. Rats again breathed atmospheric air. Ten min later, drugs or saline (control animals) were administered by intraperitoneal infusion. Twenty min after the end of the infusion, phase II was repeated.

During the experiment arterial blood pressure (left carotid artery), heart rate and ecg (lead II) were continuously recorded on a polygraph. At the end of phases I and III, and at 9 min and the end of phase II, blood Po_2 values were also determined (by a Radiometer pH/blood gas analyser, model RM 1302) on samples (100 μl) obtained from the right carotid artery via a device which permitted blood drawings without interrupting blood flow.

We selected the nitrogen/atmospheric air mixture of 70/30 on the basis of preliminary experiments performed with gas to air ratios of 50/50, 60/40, 70/30 and 80/20 using 9 rats for each group. Evaluation of the ecg patterns was performed on at least ten consecutive complexes magnified, for more precise reading, through a stereomicroscope with a scaled ocular.

Test compounds

Phosvitin (extracted and purified in our laboratories) was administered at a dose of 1 g kg^{-1}. For comparison the following compounds were also tested: DL-phosphoserine (PS) (Fluka), phosphocreatine sodium salt hexahydrate (PC) (Koch-Light) and $Na_2HPO_4.2H_2O$ (Merck), in stoichiometric amounts related to phosvitin's phosphorus content, and ATP disodium salt (Merck), in a lower dosage (75 mg kg^{-1}) because of its side effects (depression of blood pressure). Nine rats were used for each drug. Each drug was dissolved in saline and infused (Braun pump) at the rate of 0·5 ml min^{-1} (i.p.) for 6 min. The pH of each solution was adjusted to 7·4, except for Na_2HPO_4 which had a pH about 8·3.

RESULTS

In the preliminary experiments it was found that an increase in nitrogen concentration in the respiratory mixture led to a directly-related reduction in blood Po_2 levels as well as to an increase in the incidence and severity of the ecg changes. When the percentage of animals showing T-wave changes (flattening or inversion) was plotted against the blood Po_2 levels obtained with different respiratory mixtures, an inverse linear relation was observed (regression formula $r = -0{\cdot}996$; $y = 65{\cdot}737 - 0{\cdot}286$). The lower nitrogen/air ratios (50/50 and 60/40) in the respiratory mixture induced T-wave changes in a too small number of rats. Conversely, the high nitrogen concentration (80%) provoked changes in the ecg pattern in all animals; the changes, however, were always irreversible. The 70/30 gas to air ratio induced in 7 of 9 animals a greater than 85% decrease in the normal T-wave amplitude. Moreover, when the same animals underwent subsequent hypoxic episodes, the 70/30 mixture was seen to produce constant Po_2 levels and ecg changes, both as to kind and degree. Thus this ratio appeared best suited for our proposed study. It should be noted that definitive experiments with the selected mixture (70/30) in each of the groups tested gave results varying between 7/9 and 9/9 animals with the previously described T-wave changes; these changes, however, were always reversible and repeatable.

The definitive experiments showed that, in all the animals which had received phosvitin and were later subjected to phase II of the experimental design, the dramatic ecg changes of the pre-drug phase (see above) did not appear, i.e., the T-wave amplitude never dropped more than 15% of its normal value (Fig. 1). Comparing (Fisher

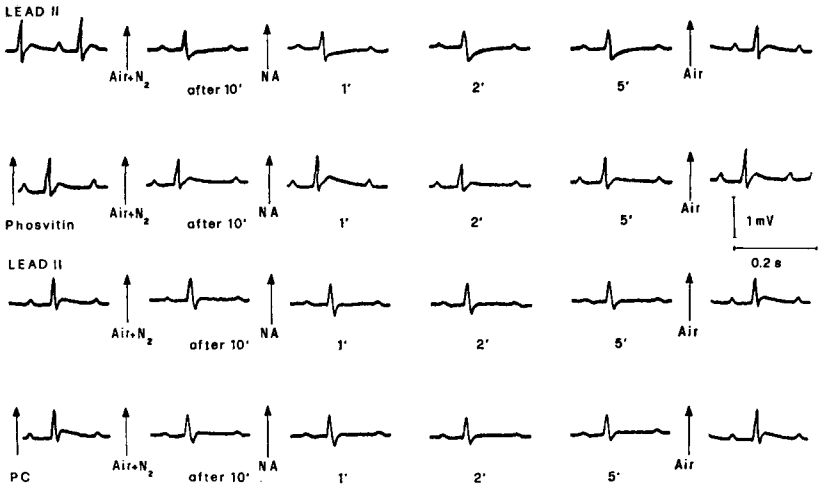

FIG. 1. Rat ecg tracings (enlarged) showing (upper) the effect of phosvitin, 1 g kg^{-1} (i.p.) and (lower) of phosphocreatine (PC), 0·5 g kg^{-1} (i.p.), on T-wave changes induced by hypoxia. Hypoxia was obtained by a mixture of 70% nitrogen (N_2) + 30% atmospheric air; following 10 min of hypoxia, 3 μg kg^{-1} of noradrenaline (NA) were administered intravenously. Five min later, hypoxia was interrupted.

exact method) the incidence of this response before (2/9 rats) and after (9/9) drug administration, a statistically significant difference ($P > 0{\cdot}01$) was clearly shown (Fig. 2).

The 15% maximum reduction value obtained in T-wave amplitude after phosvitin treatment was selected as a threshold for evaluating the possible protective action of the other compounds under the same experimental conditions. In these comparative experiments, using either PS, PC, ATP, Na_2HPO_4, or saline, no differences were found in the T-wave pattern (Fig. 1) before and after drug administration (Fig. 2).

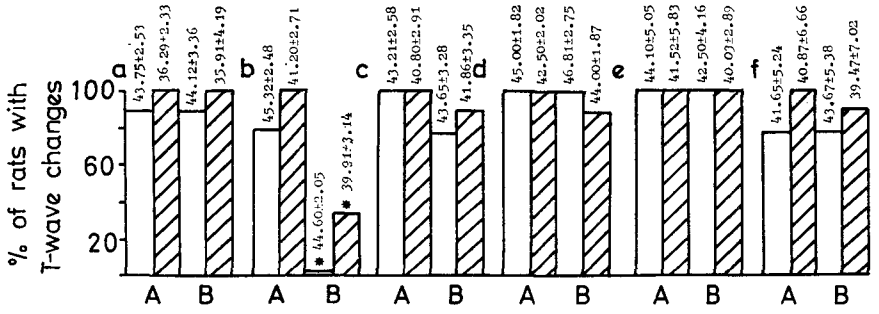

FIG. 2. The effect of phosvitin and other drugs on T-wave changes produced during hypoxia in rats. a—saline (10 ml kg^{-1}, i.p.). b—phosvitin (1 g kg^{-1}, i.p.). c—phosphocreatine (0·5 g kg^{-1}, i.p.). d—phosphoserine (0·5 g kg^{-1}, i.p.). e—ATP (75 mg kg^{-1}, i.p.). f—Na_2HPO_4 (0·5 g kg^{-1}, i.p.).

(A) Groups of 9 rats were exposed to a 70% nitrogen + 30% atmospheric air mixture and subsequently to intravenous administration of 3 μg kg^{-1} of noradrenaline during hypoxia.

(B) Each group was then given the indicated compound and re-subjected to the respiratory mixture either alone or with noradrenaline. On the ordinate are reported the percentage of rats showing T-wave changes after hypoxia (open columns) and after hypoxia + noradrenaline (hatched columns).

* $P < 0.01$ (compared with pre-drug values).

Figures above each bar indicate the mean (± s.e.) Po_2 levels.

When, during hypoxia, noradrenaline was administered to markedly increase the heart's contractile force and oxygen consumption, all the animals (9/9) showed, as expected, a slightly greater reduction in the Po_2 level and a brief increase in the severity of the T-wave changes. Phosvitin's protective effect (i.e., a 15% maximum reduction in T-wave amplitude) was, however, still observed in 6 of 9 treated rats. This value differs statistically ($P < 0{\cdot}01$) from that of the pre-drug phase (0 of 9 rats). In the drug comparison experiments, at best only one rat was similarly protected (Fig. 1 and 2).

With regard to blood pressure and heart rate, hypoxia *per se* provoked a drop in the systolic blood pressure which, after 10 min, reached the mean value of 54·40 ± 5·57mm Hg. Simultaneously, there was a mean reduction of 10·53% in the heart rate (Table 1). Injection of noradrenaline during hypoxia produced, within 15 s, an increase in the systolic pressure which reached pre-hypoxia values; the heart rate increased only slightly. Five min after noradrenaline administration, both the blood pressure and the heart rate values returned to pre-catecholamine injection levels (Table 1). Phosvitin as well as the other drugs did not influence the changes induced by hypoxia and the subsequent addition of noradrenaline in either the blood pressure or the heart rate.

P-Q and Q-S interval values, determined for all animals, remained within normal limits (Valora & Fidanza, 1963) throughout each phase of the experiment and regardless of the compound administered.

Table 1. *The effects in rats of hypoxia and of the subsequent addition of noradrenaline 3 μg kg⁻¹, (i.v.) on the blood PO_2, systolic pressure and heart rate (mean ± s.e. values).*

Parameters	Normal air	After 10 min of hypoxia	After hypoxia + noradrenaline	
			15 s	5 min
Blood Po_2 (mm Hg)	125·48 ± 3·25	44·98 ± 2·95	—	41·67 ± 3·37
Systolic pressure (mm Hg)	131·66 ± 4·79	54·40 ± 5·57	119·16 ± 6·96	53·29 ± 4·80
Heart rate (beats min⁻¹)	347·16 ± 5·85	310·60 ± 8·62	326·56 ± 6·92	309·65 ± 7·75

DISCUSSION

Severe hypoxia or ischaemia is thought to induce changes in energy production (Furchgott & De Gubareff, 1958; Michal, Naegle & others, 1959; Bing, 1965; Pool, 1969; Kübler & Spieckermann, 1970), the most important being an impairment of oxidative phosphorylation (Pool, 1970) which leads to a decrease in the myocardial concentration of ATP and to a related fall in the myocardial creatine phosphate concentration (Pool, 1970; Neely, Rovetto & others, 1973). On the other hand, some workers (see Dhalla, Yates & others, 1972 for references) found that hypoxic depression of myocardial function is not associated, except after extended hypoxia, with a decrease in myocardial ATP and creatine phosphate concentrations. A selective non-detectable depletion of ATP from a small compartment directly involved in the contraction or a defect in energy utilization by the hypoxic heart also have been suggested to explain the absence of any correlation between contractility and total ATP content (see De Jong & Goldstein, 1974 for references).

A principal property of phosvitin is its ability to be dephosphorylated by phosphoprotein-phosphatase (Sundarajan & Sarma, 1954; Rose & Heald, 1961) and to be recharged with ATP by a phosphoproteinkinase (Rabinowitz & Lipmann, 1960; Rodnight & Lavin, 1964). Thus, it is reasonable to speculate that phosvitin's protective action on the T-wave changes produced in the rat by hypoxia may be due to the release of phosphate radicals capable of direct or indirect utilization by a biological system. In this regard it is also of interest that a membrane-bound proteinkinase (Rabinowitz & Lipmann, 1960; Rabinowitz, 1962) can reversibly phosphorylate phosvitin, when this phosphoprotein is added to a biological system under equilibrium and/or other experimental conditions (see Weller & Rodnight, 1971 for references).

This hypothesis concerning phosvitin's mechanism of action does not exclude other possibilities such as a depressive effect on the cardiac work, an increase in the coronary flow or an impaired release of endogenous catecholamines. However, our experimental data from tests performed on isolated guinea-pig ileum stimulated by $BaCl_2$ and on rat aortic strips (Caprino, Borrelli & others, 1976) render it improbable that phosvitin has an effect on coronary blood flow. It is also important to note that phosvitin did not influence the normal blood pressure in the animals nor did it modify either the pressure responses to catecholamines or the cat nictitating membrane responses to electrical stimulation (unpublished observations).

Phosvitin's protective action of the ecg changes after noradrenaline injection during hypoxia, in the face of the obvious implications of administering noradrenaline to hypoxic animals and apart from other possible interpretations, could derive from the same mechanism by which it acts on hypoxia alone. The effect of nordarenaline may be viewed simply as aggravating temporarily the hypoxia produced by the 70/30 respiratory mixture, thus yielding an hypoxic effect similar to that of the 80/20 ratio, but reversible.

If our hypothesis concerning phosvitin's mechanism of action is valid, one may ask why ATP, PC and PS are not similarly effective under the same experimental conditions. One might conjecture that exogenous PC and ATP could follow the same metabolic pathway as the endogenous forms, thus becoming unsuitable as an energy supply. Under the present experimental conditions, ATP could hypothetically be continuously split to adenosine to obtain a coronary-dilating effect (Rubio, Berne & Dobson, 1973). In support of this hypothesis are the findings that allopurinol, which impairs the splitting of high energy phosphate compounds to adenosine, inosine and other related compounds, was found to prevent electrocardiographic S-T changes of ischaemic origin and biochemical changes induced by coronary artery ligation in dogs and sheep (De Wall & others, 1971).

At present, even if phosphoserine's inactivity remains unclear, it should be noted that the phosphate transfer catalysed by proteinkinases only occurs in proteins which contain adjacent phosphoserine residues (reactive sites) arranged in a typical pattern such as is found in phosvitin (Rabinowitz & Lipmann, 1960).

It is evident, however, that additional clarifying investigations are needed to determine the precise mechanism of phosvitin's protective action on hypoxic ecg changes.

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