SHORT-TERM EFFECTS OF OUABAIN ON ENERGY-RICH, GLYCOLYTIC AND CITRIC-ACID-CYCLE INTERMEDIATES IN FROG HEART

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According to recent investigations (Furchgott and de Gubareff, 1958; Lee and Yu, 1963), direct interaction with ion translocation mechanisms -the most important effect of cardiac glycosides (Repke and Portius, 1963)- does not entail significant changes in the levels of energy-rich compounds. In the present communication the levels of 18 metabolites (P-creatine, creatine, ATP, ADP, AMP, glucose, glucose-1-P, glucose-6-P, fructose-6-P, fructose diphosphate, dihydroxyacetone-P, glyceraldehyde-P, α-glycero-P, lactate, pyruvate, oxaloacetate, malate, α-ketoglutarate) were determined in frog hearts. The frogs were injected with ouabain and the hearts were quick-frozen 50 sec later, prior to the appearence of toxic signs.

Analysis of the results by means of the stepwise discriminant analysis (Dixon, 1964) shows that a significant discrimination exists between the control and the ouabain-treated hearts; the pattern of the changes permits to draw indication on the metabolic effects of ouabain.

Materials and Methods. Spring frogs of both sexes, weighing 15 to 25 g were used. After spinalization and opening of the chest, the frogs were injected directly in the vena cava inferior with 1 ml 0.1% ouabain dissolved in Ringer-Frog solution. At the first sign of arrhythmia preceding systolic contracture, the hearts were quick-frozen in situ by means of metal tongs cooled to the temperature of liquid nitrogen. Control frogs of the same batch were injected with 1 ml Ringer-Frog solution and the hearts quick-frozen after 50 sec. All the above procedures were completed within two days. Twelve groups of 25 frogs each were used. Each group of 25 frozen hearts was powdered in a percussion mortar chilled with liquid

nitrogen and extracted with 6% $HClO_4$. The substrates were determined with enzymatic methods according to Bergmeyer (1963); some of the modifications suggested by Lowry et al. (1964) were taken into account.

Stepwise discriminant analysis was carried out on the 6 groups of control and the 6 groups of treated hearts with the program BMD 07 M (Dixon, 1964) and an IBM 7094 computer.

Results. Discriminant analysis shows that the ouabain-treated animals are different from the controls. The analysis was performed either on the above mentioned 18 levels of metabolites or on 33 parameters -the previous plus 15 derived parameters (Table 1)- and in both cases brings about the discrimination. The difference is statistically significant at F 0.01 level and the two sets of groups do not overlap when the canonical analysis is used to represent the data in a plane (Figure 1).

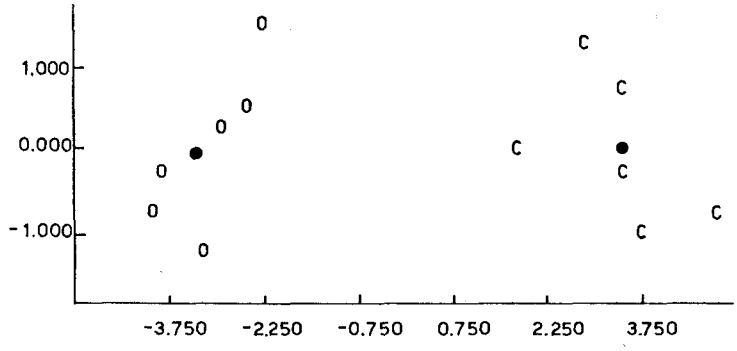

Figure 1. Results of the canonical analysis of the data obtained on 33 parameters from ouabain-treated (O) (6 groups) and from control (C) (6 groups) frog hearts. Values on abscissa and on ordinate: first and second canonical variable, which identify each group. For details, see Dixon (1964). ● indicates the mean value between the groups for both experimental conditions.

Table 1. Effect of ouabain on the levels of 18 metabolites and on the values of the 15 calculated parameters. Mean values and standard deviations, umoles/g wet weight.

	Control	Treated
1. Glucose-1-P (G1P)	23.28 + 4.73	20.35 + 10.34
2. Glucose	987.2 + 123.5	916.30 + 95.6
3. Glucose-6-P (G6P)	244.7 + 17.71	248.17 + 12.98
4. Fructose-6-P (F6P)	47.68 + 4.56	49.72 + 4.96
5. Fructose diphosphate (FDP)	60.25 + 9.95	68.98 + 11.79
6. Dihydroxyacetone-P (DAP)	23.75 + 2.73	31.20 + 4.58
7. Glyceraldehyde-P GAP)	2.27 + 0.44	1.38 + 0.54
8. α-Glycero-P (α-GP)	39.57 + 11.79	58.85 + 6.97
9. Pyruvate (P)	73.23 + 7.21	71.60 + 8.02
10. Lactate (L)	2430 + 1038	2257 + 874.7
11. Malate (M)	122.5 + 20.89	123.83 + 13.04
12. Oxaloacetate (OAA)	3.02 + 0.95	2.45 + 0.37
13. α-Ketoglutarate	56.88 + 15.86	47.95 + 10.70
14. P-Creatine (PC)	2102 + 114.4	1918 + 45.79
15. Creatine (Cr)	2322 + 170.2	2365 + 219.1
16. ATP	2673 + 58.88	2530 + 104.5
17. ADP	720.3 + 42.77	739.7 + 62.0
18. AMP	358.5 + 87.02	313.17 + 81.81
19. G1P / G6P	0.1 + 0.02	0.082 + 0.041
20. F6P / G6P	0.195 + 0.01	0.2 + 0.014
21. DAP / GAP	10.83 + 2.42	25.63 + 10.04
22. α-GP / DAP	1.68 + 0.52	1.9 + 0.15
23. FDP x ADP / F6P x ATP	0.478 + 0.097	0.55 + 0.101
24. DAP / FDP	9.75 + 3.25	14.25 + 2.69
25. DAP x GAP / FDP	0.937 + 0.368	0.633 + 0.279
26. L / P	32.82 + 11.5	31.17 + 10.32
27. M / OAA	45.17 + 20.22	51.5 + 9.07
28. PC / Cr	0.908 + 0.068	0.817 + 0.091
29. ATP / ADP	3.71 + 0.26	3.44 + 0.24
30. ATP x AMP / ADP^2	1.82 + 0.33	1.84 + 0.33
31. PC + Cr	4432 + 236	4283 + 185
32. ATP + ADP + AMP	3742 + 99.3	3583 + 207
33. Cr x ATP / PC x ADP	4.1 + 0.47	4.22 + 0.31

The sequence by which the parameters are included in the discrimination process is: P-creatine, glucose, glucose-1-P, dihydroxyacetone-P, malate, fructose diphosphate, AMP, fructose-6-P, glucose-6-P and creatine (18 parameters analysis); and: mass-action ratios of the phosphoglucomutase, aldolase and phosphohexose isomerase reactions, the sums: ATP+ADP+AMP, and P-creatine+creatine (33 parameters analysis).

Ouabain affected both metabolites of the glycolytic pathway and compounds such as P-creatine, creatine and AMP. A crossover point may be seen between glucose, glucose-1-P and

glucose-6-P, in correspondence with the glucokinase-phosphogluco= mutase steps (Figure 2). The redox ratios: α-glycero-P/dihydroxyace= tone-P, lactate/pyruvate and malate/oxaloacetate were not affected by ouabain, nor were the substrates themselves, with the exception of malate. While ATP and ADP were unaffected, the decrease of P-creatine and AMP, as well as the increase of creatine were important in the discrimination.

Discussion. Multivariate analysis shows a statistically significant difference between the normal and the ouabain-treated hearts and proves to be a powerful tool for discriminating two populations of metabolically correlated data. The occurrence of a crossover point at the glucose phosphorylation site indicates that ouabain brings about an

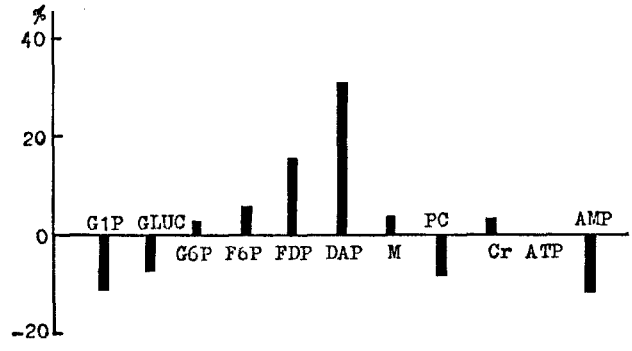

Figure 2. Percent changes, relative to the control group, of the substrates which discriminate the ouabain-treated from the control hearts. Abbreviations used: see Table 1.

increase of the rate of glucose entry into the cell. The nature of the ouabain effect on glucose transport is a controversial question. Contrary to data of Clausen (1966), who reports a slowing effect in rat diaphragm muscle, our results agree with the work of Segre (1949), of Kien et al. (1960) and of Wollenberger et al. (1962). The constancy of the redox potentials of the cytoplasmic NAD-$NADH_2$-system is in contrast with the finding of Rossini et al. (1966 a), who observed in the ouabain-treated low-adapting neuron of the stretch organ of the crayfish, by means of a microfluorimetric technique, an increase of the pyridine-nucleotide reduction. This was possibly due to the formation of $NADPH_2$, in accordance with the results of Wollenberger (1953), who noticed a ouabain

stimulation of the direct glucose shunt. By injecting in the vena cava the solution at the rate of 1 ml/min a washing effect on such easily diffusible substrates as lactate and pyruvate has been observed (Arese and Rossini, unpublished). This could mask a change in the lactate/pyruvate ratio. In fact, an analysis of the metabolite changes occurring 30 sec after the ouabain injection shows changes in the redox ratios: these results therefore need to be confirmed. The changes of P-creatine, creatine, and AMP are consistent with recent results of Rossini et al. (1966 b), which demonstrate an unexpected activation of the Na^+-K^+-activated ATP-ase of the frog heart caused by ouabain in a wide range of concentrations. The constancy of ATP and ADP is readily explained by the buffering effect of P-creatine, while the decrease of AMP can be caused by the enhancement of adenosine deaminase activity. Further experimental results, showing short-term effects of perfusion with high Ca^{++} and low K^+ Ringer solutions on the same metabolites in the frog heart are to be published elsewhere.

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