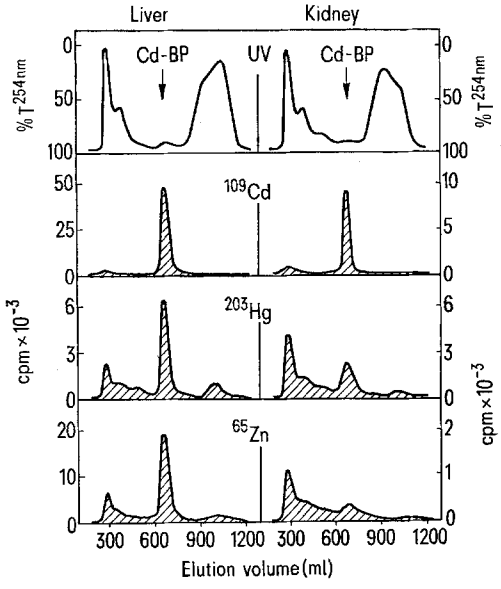
Distribution of ^{109}Cd, ^{203}Hg and ^{65}Zn, in liver and kidney of *Carassius auratus*, 24 h after i.p. injection of 30 nmoles of each labelled metal (% of the dose)

	109 Cadmium	203 Mercury	65 Zinc
Liver			
Total	27.5	6.9	8.1
Cytosol	26.3	2.3	4.9
Cd-BP	24.5	1.2	3.2
Kidney			
Total	7.4	4.0	1.6
Cytosol	6.1	1.3	0.3
Cd-BP	5.2	0.5	0.03



Gel filtration on Sephadex G-75 of the cytosol of liver and kidney of goldfish injected with 30 nmoles of ^{109}Cd, ^{203}Hg and ^{65}Zn, 24 h before the sacrifice.

the cytosol by the chromatographic procedure of SHAIK and LUCIIS[11] on a Sephadex G-75 columns, 100 × 3 cm, equilibrated with *Tris*-Cl buffer 10 m*M* at pH 8.2. The UV-transmission of the eluate was continuously monitored at 254 nm, and the radioactivities measured in the collected fractions (5 ml).

The Figure shows the elution profiles obtained from the gel-filtration of the liver and the kidney cytosol. Besides the cadmium, there was also mercury and zinc incorporated into Cd-BP, both in the liver and in the kidney. In these 2 organs, all the cadmium radioacvity was recovered in the Cd-BP fractions, while for mercury and zinc the radioactivity was found both in the Cd-BP and the higher molecular weight proteins.

The results reported in the Table show the distribution of ^{109}Cd, ^{203}Hg and ^{65}Zn in the liver and in the kidney. It was found that most cadmium was present in the cytosol of the liver, and that it was incorporated into the Cd-BP. In the kidney, the fraction bound to the Cd-BP represents only 70% of that found in the total organ. The results for Hg indicate, in comparison to Cd, lower proportions found in the cytosol (around $^1/_3$) and in the Cd-BP (12 to 17%). These proportions appear similar for liver and kidney.

As far as the distribution of ^{65}Zn is concerned, a strong difference was observed between liver and kidney. In kidney, the Cd-BP incorporates much less zinc (1.9%) than in liver (39.5%). This suggests the possibility of the presence in fish of an hepatic zinc-binding protein, with characteristics similar to the Cd-BP, as reported for the human liver by BÜHLER and KÄGI[12]. Although some differences were observed between the 3 metals in their affinity for the Cd-BP of liver and kidney, the results reported indicate that in fish, as in mammals, this metal-binding protein represents an important binding-site for the II B elements of the periodic table. Since cadmium stimulates the synthesis of the Cd-BP, the incorporation of different metals in the same component would explain some interactions observed in the metabolism of such metals in fish exposed to polluted water[13].

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The Response of Ca-mediated Action Potentials and Contractile Activity in Mammalian Ventricular Myocardium Towards Alkalosis

M. KOHLHARDT and K. HAAP

Physiologisches Institut der Universität, Hermann-Herder-Strasse 7, D–78 Freiburg im Breisgau (German Federal Republic, BRD), 19 September 1975.

Summary. Alkalosis (pH 7.8) produced by reduction of CO_2 concentration augmented both upstroke velocity of Ca action potentials and isometric contractile force of mammalian heart muscle. If the increase of pH to 7.8 was achieved by a raise of HCO_3 concentration (with simultaneous reduction of CO_2 concentration), the positive inotropic response was not accompanied by an augmented Ca current. Obviously, the well-known positive inotropic effect of alkalosis does not only depend upon the enhancement of transmembrane Ca influx during excitation, but can be mediated alone by affecting intracellular Ca movements as well.

It is a well-known fact that changes of pH in the extracellular fluid induce alterations of contractile activity of the myocardial cell. Thus, a decrease of the H concentration exerts a positive inotropic effect[1]. This reflects a larger amount of activator Ca available at the myofibrils which could be caused by a promoting action of alkalosis upon Ca release from stores (NAKAMARU and SCHWARTZ[2]) if changes of extracellular pH lead to concomitant alterations of the H concentration in the cell. On the other hand, it would be conceivable that alkalosis enhances transmembrane Ca inward current. In order to obtain a more precise insight in the mechanism under-

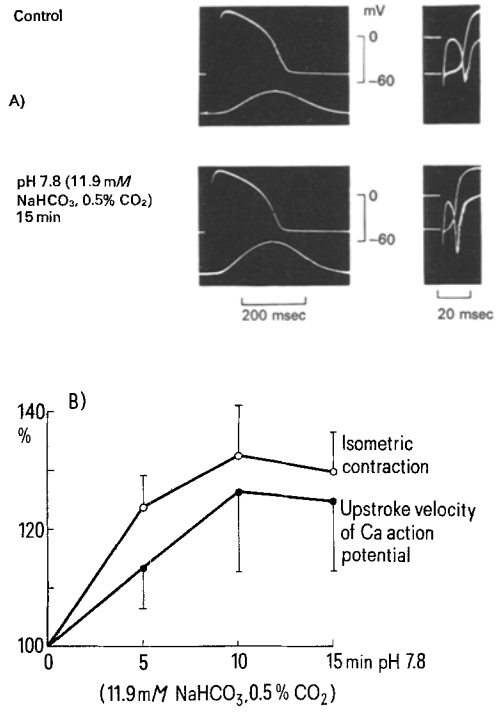

Fig. 1. A. Left panels: registration of the Ca mediated action potential and the isometric contraction of a papillary muscle from the right ventricle of a cat. Right panels: Differentiation of the upstroke phase of the Ca action potential. The upper records represent the control values at pH 7.4, the lower records were obtained 15 min after the increase of pH to 7.8 by reducing the CO_2 concentration to 0.5%. Continuous impalement of the microelectrode.

B. Time course of the effect of pH 7.8 (11.9 m*M* HCO_3, 0.5% CO_2) upon upstroke velocity of the Ca action potential (filled circles) and isometric contraction (open circles). Each point represents the mean value from 10 experiments (vertical bars: standard deviation).

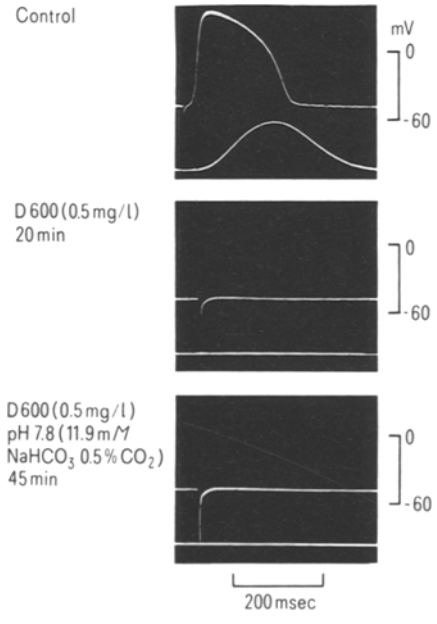

Fig. 2. Registration of Ca action potential and isometric contraction of a papillary muscle before (upper panel), 20 min after the addition of 0.5 mg/l D600 (middle panel), and after switching to a D600 – containing medium with a pH of 7.8 (11.9 m*M* HCO_3. 0.5% CO_2). Continuous impalement of the microelectrode.

lying the positive inotropic action of alkalosis, the changes of the Ca mediated action potential and the contractile activity in papillary muscles of cats were studied following an increase of extracellular pH from 7.4 to 7.8.

Methods. According the procedure described by MASCHER[3], Ca mediated action potentials were elicited after inactivation of the fast Na system of the membrane which was achieved by decreasing the resting potential to −50 mV using K-rich (15 m*M*) Tyrode solution (NaCl 137 m*M*, $CaCl_2$ 2 m*M*, $NaHCO_3$ 11.9 m*M*, NaH_2PO_4 0.42 m*M*, glucose 10 m*M*; bubbled with carbogen (97% O_2, 3% CO_2, temperature 34 °C). For the registration of the action potentials KCl-filled microelectrodes were used. The rate of rise was considered to be representative for the (Ca carried) slow inward current. Stimulation frequency 60/min.

Results and discussion. In a first type of experiments, the pH of 7.8 was attained by decreasing the CO_2 concentration from 3% to 0.5%. As shown in Figure 1a, 15 min after switching to this alkalotic Tyrode solution an increase of upstroke velocity of the Ca mediated action potential occurred which amounted to 33% in this experiment. Compared with the control values at pH 7.4, resting potential, overshoot and duration (at the level of 50% and 90% repolarization) of the Ca action potential remained unaffected. As expected, the force of isometric contraction went up, in this case by 40%. In a quantitative respect, this type of alkalosis produced a nearly identical response of both upstroke velocity and isometric contraction (Figure 1b). Within 15 min, a gradual increase appeared, and finally a mean augmentation of upstroke velocity by 24.7% and of contractile force by 29.7% was obtained which does not differ significantly from each other. This increase of upstroke velocity of the Ca action potential reflects an enlargement of the transmembrane slow inward current. As recently suggested by DROUIN and NEUMCKE[4], the inward movements of Na ions through the fast membrane channel presupposes their binding to specific negative charges which become protonated in the presence of low extracellular pH thus blocking the fast Na current. Consequently, the rise in pH should lead to a reduced protonation of negatively charged groups. If the transport elements of the slow channel react in the same way, an increase in transmembrane Ca current occurs.

It was now the question whether this alkalosis could neutralize the action of D600: 5 min after adding this compound to the Tyrode solution (pH 7.4) the known decrease of upstroke velocity and overshoot of the Ca action potential appeared accompanied by a diminution of contractile force (TRITTHART et al.[5]). As shown in Figure 2, after a time of exposure of 20 min to D600 (0.5 mg/l) the Ca action potential and the contractile activity disappeared indicating the blockade of the slow membrane channel (KOHLHARDT et al.[6]). This blocking effect of D600 was not neutralized by alkalosis which may be explained by the relatively small increase of slow channel conductance due to pH 7.8 so that the strong inhibitory D600 action could not be overcome.

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The results described above clearly demonstrate that the augmentation of contractile force in the presence of pH 7.8 depends mainly upon the enhanced transmembrane Ca influx during excitation. In order to see whether alkalosis exerts its positive inotropic effect on principle via an increase of Ca current, in a second type of experiments pH was elevated by means of a stronger HCO_3 concentration (23.8 m*M*) with simultaneous reduction of the CO_2 concentration to 1%. Figure 3 shows the evaluation of these experiments. Again, within 15 min an increase of isometric contraction force occurred which amounted to 28.7% on an average. A longer time of exposure to the alkalotic Tyrode solution had no additional effect. However, a clearly different response of the Ca action potential was obtained; 10 min after switching to this medium a slight decrease of its upstroke velocity by 14.5% appeared. It was followed by a rise attaining the control values of upstroke velocity 5 min later. Obviously, the increase of contractile force is not accompanied with proportional changes in transmembrane Ca current. The same phenomenon was found under the influence of cardiac glycosides (THYRUM[7]; NAWRATH et al.[8]), which proves that an enlargement of Ca influx during excitation has not to be considered as an essential prerequisite for the positive inotropic action in heart muscle. Rather the underlying increase in activator Ca can be caused predominantly by changes of intracellular Ca movements as well. In the case of extracellular alkalosis (produced by increased HCO_3 and reduced CO_2 concentration) the augmented contractile activity seems to results from promoting the Ca release from stores probably induced by concomitant changes of the intracellular H concentration. If this leads to a rise in intracellular free Ca, a plausible explanation for the unexpected cessation of the augmentation of Ca currrent becomes available.

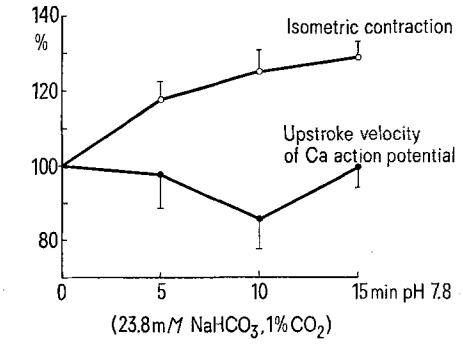

Fig. 3. Changes of upstroke velocity of Ca action potential (filled circels) and isometric contraction (open circles) following perfusion with a 23.8 m*M* HCO_3- and 1% CO_2-containing medium (pH 7.8). Each point represents the mean value from 5 experiments (vertical bars: standard deviation).

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5-Thio-D-Glucose: *o*-Diphenoloxidase Inhibition as its Mechanism of Action

K. PRABHAKARAN

U.S. Public Health Service Hospital, Carville (Louisiana 70721, USA), 1 September 1975.

Summary. 5-Thio-D-glucose completely inhibited *o*-diphenoloxidase from animal as well as plant sources. It has been reported that thioglucose suppresses spermatogenesis in mice and also insect metamorphosis, probably through inhibition of glucose transport. Inhibition of *o*-diphenoloxidase (which is active in spermatozoa and insect larvae) is suggested as an alternative mechanism of action of thioglucose.

This communication deals with a possible mechanism of action of 5-thio-D-glucose, which has been reported to be a unique male contraceptive[1]. In thioglucose, which was synthesized in 1962, the ring oxygen of glucose is replaced by a sulfur atom[2]. Feeding of male mice with the compound resulted in testicular atrophy and total inhibition of spermatogenesis[1]. The effect was fully reversible on withdrawing the drug. When thioglucose was fed to larvae of *Drosophila melanogaster*, metamorphosis of the larvae was suppressed[3]. Although the drug inhibits competitively the active transport of D-glucose across cell membranes[4], the exact mechanism of action of thioglucose on spermatogenesis or insect metamorphosis has not yet been established.

It has been pointed out that, since glucose is a major energy source for brain metabolism, an antagonist of glucose could have serious side effects[5]. However, no acute toxic effects were observed in experimental animals given large doses of the drug, and LD_{50} of thioglucose was quite high (14 g/kg body weight)[4]. Evidently, the compound might have some other mode of action as well, besides inhibiting glucose transport. Spermatozoa contain an enzyme which actively oxidizes dopa to pigment[7–9], and sulfur-containing compounds are known to be inhibitors of *o*-diphenoloxidase (tyrosinase) (*o*-diphenol: oxygen oxidoreductase)[10]. Moreover, tryosinase is known to have an important role in insect development[11].

Methods. To study the effect of 5-thio-D-glucose on *o*-diphenoloxidase, the oxidation of dopa (3,4-dihydroxyphenylalanine) by cultured melanoma cells and by lyophilized mushroom tyrosinase was tested with and without the drug. Mushroom tyrosinase and 5-thio-glucose

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