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Preliminary Findings on Calmodulin-stimulated Ca^{2+}-ATPase of Erythrocyte Ghosts in Psychotic Patients*, **

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Summary. Kinetic properties of the calmodulin-stimulated erythrocyte ghost Ca^{2+}-ATPase seem to be altered in some sub-groups of affective and schizophrenic psychoses. The subgroup of affective disorders concerned mostly unipolar manic and bipolar psychoses with predominantly manic episodes. In the corresponding cases from schizophrenics hyper- and parakinetic basic syndromes were predominantly diagnosed. An evaluation of our preliminary results was undertaken in connection with our biochemical hypothesis on possible alterations in the regulation of Ca^{2+} concentrations and Ca^{2+}-calmodulin-mediated processes under certain psychotic conditions.

Key words: Ca^{2+}-ATPase – Calmodulin – Erythrocyte membranes – Psychoses

Previous hypotheses on the biochemical basis of psychoses have focused on those process changes within the chemical transmission chain of synapses which immediately concern the transmitters themselves and/or their post-synaptic receptors, i.e. changes in synthesis, catabolism, release, re-uptake, receptor sensitivity and transmitter-receptor interaction. However, in the light of our constantly increasing information on the regulatory effect Ca^{2+} has on a series of these processes, especially when combined with calmodulin and other modulator proteins, we came to the conclusion that this ion should by all means be included in biochemical concepts of psychoses. In support of this conclusion, reference is made to the fact that some anti-psychotic drugs are Ca^{2+} antagonists and as such assumed to inhibit calmodulin effects [2, 5, 8]. The conceptual spectrum of possible processes, however, should not be restricted to Ca^{2+}-mediated reactions, but should also include the regulating mechanisms of Ca^{2+} concentrations in resting and excited synapses. Alterations under psychotic conditions in the Ca^{2+} influx and in the removal processes of intracellular Ca^{2+} must also be taken into consideration.

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We have been working in the field of calmodulin-stimulated Ca^{2+}-ATPase for about 2 years, basing our research on the following hypotheses:

Knowing that calmodulin-stimulated Ca^{2+}-ATPase occurs ubiquitously in cellular plasma membranes, but having no neuronal cell material from patients and/or control persons, we started by investigating erythrocyte ghosts. The physiological role of this ATPase in regulating Ca^{2+} levels in nerve terminals and erythrocytes seems to be analogous, although differences between the enzyme from the two sources have been reported [1]; they concerned in particular the composition of enzyme-associated lipids and proteins as well as the various types of calmodulin embedding. Despite these differences, however, it may be assumed that possible changes of the Ca^{2+}-ATPase in neuronal plasma membranes are reflected analogously in those of erythrocyte ghosts.

Presented here are some preliminary results on changes in kinetic parameters of the ghosts' ATPase from 39 psychotic patients and 12 control persons. The patients had either stopped taking drugs 4 weeks before the investigations or—if the psychotic disorder had occurred for the first time—had not been treated at all. Diagnosis of the 39 patients in accordance with the "International Classification of Diseases" (ICD-9) revealed the following:

2 unipolar manic patients (296.0), 7 bipolar patients with predominantly manic episodes (296.2), 7 unipolar depressive patients (296.1), 9 bipolar patients with predominantly depressive episodes (296.3), 2 schizophrenics with a chronic progressive course and 12 with acute episode (295). Additionally, to correlate biochemical findings with clinical diagnoses, a further classification according to basic syndromes, i.e. hypo-, dys-, hyper-, and parakinetic syndromes, was made [4]. The procedures for the preparation of calmodulin-depleted erythrocyte ghosts by isotonic haemolysis in the presence of saponin, assay of Ca^{2+}-ATPase and calculation of free Ca^{2+} concentrations in the test medium were carried out as described elsewhere [3]. For maximum stimulation of the enzyme 2 mg/l calmodulin and 3 m*M* Mg^{2+} were generally used in the test medium.

According to the kinetic parameters we evaluated, the Ca^{2+} dependence of the Ca^{2+}-ATPase in the presence and in the absence of calmodulin provided the most suitable data for correlation attempts to psychotic syndromes. As seen in Fig. 1, we could essentially categorize two types of responses of Ca^{2+}-ATPase to maximum stimulation by calmodulin

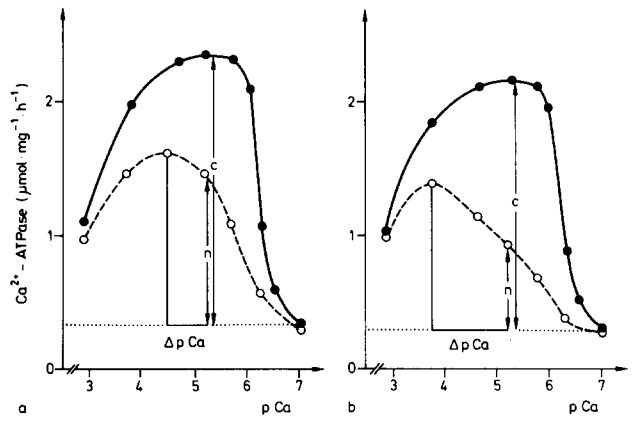

Fig. 1a, b. Different types of responses of Ca^{2+}-ATPase to maximum stimulation by calmodulin: Small (**a**) and large differences (**b**) between the pCa optima with and without calmodulin (ΔpCa). Symbols: c = calmodulin-stimulated and n = basic activity at the pCa optimum of the first; $Q = c/n$. (○——○) Basic activity without calmodulin; (●——●) calmodulin-stimulated activity. Equations of the regression lines, standard deviations and correlation coefficients (Ps = psychoses, Co = controls):

$$y^{Co} = 0.76x - 0.64;\quad s_{xy}^{Co} = \pm 0.32;\quad r^{Co} = 0.706$$
$$y^{Ps} = 0.87x - 0.67;\quad s_{xy}^{Ps} = \pm 0.40;\quad r^{Ps} = 0.705$$

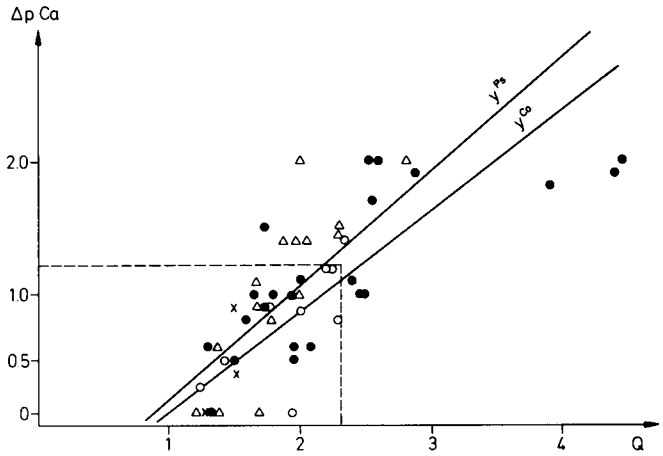

Fig. 2. Correlation between ΔpCa and Q. Symbols: (○) male controls; (x) female controls; (●) male psychoses; (△) female psychoses

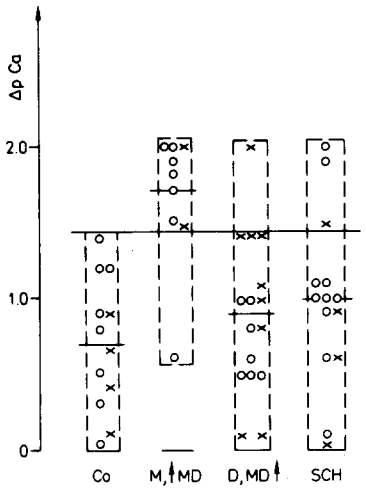

Fig. 3. Relationships between ΔpCa and clinical diagnoses. Symbols (○) male probands; (x) female probands

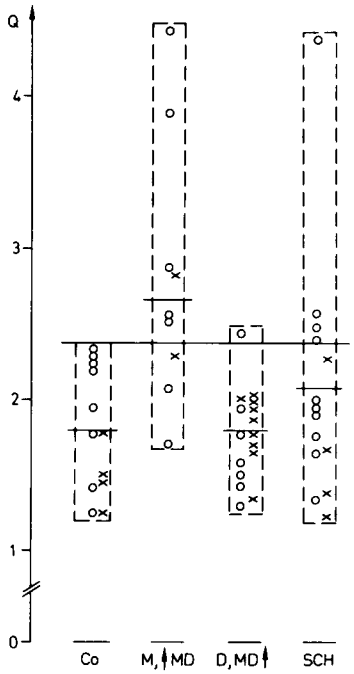

Fig. 4. Relationships between Q and clinical diagnoses. Symbols see Fig. 3

within the range of free Ca^{2+} from 10^{-7} to $10^{-3}M$ (corresponding to pCa from 7 to 3). One type is characterized by a relatively small (Fig. 1a), the other type by a larger distance between the pCa optima of both the basic and the calmodulin-stimulated ATPase (Fig. 1b). This pCa difference (ΔpCa) was a decisive feature in correlating parameters from the individual kinetics of all probands. From the derived parameters, the quotient Q of the calmodulin-stimulated (c) to the non-stimulated basic Ca^{2+}-ATPase activity (n) at the pCa optimum of c seems to be most useful (designated graphically in Fig. 1 by arrows).

As shown in Fig. 2, ΔpCa and Q are well correlated in the controls and in the whole group of psychotics. The correlation coefficients are nearly identical with one another, and the regression lines only differ insignificantly. The value pairs of the controls are enclosed in a rectangle bounded by lines corresponding to $\Delta pCa \leqq 1.1$ and $Q \leqq 2.35$. This area also includes a part of pairs of psychotic patients. However, a considerable number of pairs of the latter one localized outside this area.

The attempts at correlation with the clinical diagnoses led to the interesting results seen in Fig. 3 in the case of ΔpCa and in Fig. 4 in the case of Q: In general, unipolar manic and bipolar psychoses with predominantly manic episodes showed that the Ca^{2+}-ATPase parameters almost completely (ΔpCa), or in the greater part (Q), exceeded those of the controls. This enabled us to summarize both sub-groups (M,↑MD). The same procedure was employed in the cases of unipolar depressive and bipolar psychoses with predominantly depressive episodes (D,MD↑) which did not deviate from the control values. The group of schizophrenics (SCH) did not permit a further differentiation of its sub-groups, because the cases

with elevated values belonged to chronic as well as to acute courses. However, in both the chronic and acute schizophrenics with elevated Q and/or ΔpCa hyper- and parakinetic basic syndromes were predominantly diagnosed.

To summarize, some sub-groups of affective and possibly of schizophrenic psychoses seem to exist with altered properties of the calmodulin-stimulated Ca^{2+}-ATPase of their erythrocyte membranes. The first sub-group includes affective psychoses with predominantly or exclusively manic syndromes, the other includes some distinct hyper- or parakinetic schizophrenics. These results, although obtained from first tentative tests and therefore not permitting statistical evaluation and final conclusions, appear to confirm in principle the validity of our hypothesis mentioned above. More profound statements such as age and sex differences, treatment effects and individual courses during remission are not possible at present. The numerous open problems will be investigated in the future. In addition, recent publications from Linnoila and Mac Donald also support our conceptual aspects [6, 7]. This team at first described a correlation of the basic ghosts Ca^{2+}-ATPase (without calmodulin) with manic and depressive syndromes [6], and later a greater variability of this enzyme to calmodulin stimulation in 12 patients with bipolar affective disorders in comparison with age- and sex-matched controls. Although there were differences in methodological aspects of the kinetic experiments and of the evaluation of the results between these authors and us, some corresponding conclusions may be drawn, indicating their complementary nature.

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