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Partially Phosphorylated Phosphorylase in the Rat Heart After β-Receptor Stimulation in vivo

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Summary

The formation of the phosphorylase *ab* hybrid and its further transformation into phosphorylase *a* has been demonstrated in the rat heart after different periods of i.v. isoproterenol administration. Phosphorylase *ab* hybrid was determined in the presence of AMP and/or caffeine. Only the partially phosphorylated phosphorylase was found in the control rat hearts and its activity was 30 % of the total phosphorylase. The phosphorylase *ab* hybrid was disclosed particularly after small isoproterenol doses (0.031–0.062 $\mu g.kg^{-1}$) and at short time interval (15 s) after its administration. Higher isoproterenol doses (0.25–0.5 $\mu g.kg^{-1}$) changed the partially phosphorylated phosphorylase to phosphorylase *a* (58 %) after a longer time interval (40 s). The phosphorylase *ab* hybrid was revealed even at the maximal rate of stimulation. The formation of the phosphorylase *ab* hybrid in the rat heart *in vivo* appears to be of physiological significance. Our results confirmed the earlier suggestion that the −AMP/+AMP activity ratio reflects the percentage proportion of the phosphorylated subunits of phosphorylase but not of the activated phosphorylase molecules.

Key words

Glycogen phosphorylase – Phosphorylase *ab* hybrid – Isoproterenol – Protein phosphorylation – Rat heart

Introduction

Stimulatory effect of catecholamines on the phosphorylase activity in the heart is well documented (Gardner and Allen 1977, Namm and Mayer 1981, Buczek-Thomas *et al.* 1992). As a result of stimulation the nonphosphorylated phosphorylase *b* is transformed into totally phosphorylated phosphorylase *a*. Phosphorylase *b* exhibits catalytic activity only in the presence of AMP, while phosphorylase *a* is active by itself (Larner 1976, Dombrádi 1981, Walsh *et al.* 1991).

The phosphorylase activity ratio −AMP/+AMP often reached a maximum value of only about 0.5. The existence of the phosphorylase *ab* hybrid in the heart is not currently considered in spite of the fact that the activity ratio is highly suggestive. Vereb *et al.* (1986) demonstrated the formation of the phosphorylase *ab* hybrid both *in vitro* and in perfused rat hearts and introduced a procedure for calculating its amount.

The aim of the present study was to show the formation of the phosphorylase *ab* hybrid in the rat heart after β-receptor stimulation *in vivo*. Until now, the existence of the heart phosphorylase *ab* hybrid has not been studied *in vivo*. The formation of the phosphorylase *ab* hybrid simultaneously with phosphorylase *a* in rat hearts after different isoproterenol doses and at various time intervals suggests its physiological significance.

Methods

Male Wistar rats weighing 300–400 g were used in the experiments. The animals were fed a standard diet and water was supplied *ad libitum* throughout the experiment. The rats were anaesthetized with intraperitoneal injection of pentobarbital (45 $mg.kg^{-1}$). In order to attain standard metabolic conditions the animals were artificially respirated for 10 min under open chest conditions. Isoproterenol was then administered intravenously in doses of 0.031–0.5 $\mu g.kg^{-1}$. The hearts were freeze-

clamped with Wollenberger tongs (prechilled with liquid nitrogen) 15 s or 40 s after isoproterenol administration. The frozen tissue from the whole heart was powdered under liquid nitrogen and homogenized with a solution containing 20 mM NaF, 1 mM EDTA pH=7 (40 mg tissue.ml^{-1}). After centrifugation (52.3x10^3 m.s^{-2}, 15 min) the supernatant was repeatedly treated with charcoal (3 mg/40 mg wet weight).

Phosphorylase activity estimations were performed according to Cori and Illingworth (1956). The reaction mixture composition was modified as follows: a) no addition (−AMP), b) with 1 mM AMP(+AMP) and c) 1 mM AMP + 5 mM caffeine (AMP + caffeine). The unit of enzyme activity was defined as the amount of enzyme which liberates 1 μmol P_i from glucose-1-phosphate per min. Liberated amount of Pi was determined according to Goldenberg and Fernandez (1966). Total phosphorylase was determined as the activity measured in the presence of 1 mM AMP. The phosphorylase *ab* hybrid and phosphorylase *a* were calculated from the activities estimated in the presence of 1 mM AMP + 5 mM caffeine and without it as described by Vereb *et al.* (1986). Taking into consideration that the activity of the phosphorylase *ab* hybrid is doubled in the presence of AMP + caffeine, the value of *ab*/2 was calculated as the difference of the activities without AMP and corrected for (20 %) AMP + caffeine. The amount of the phosphorylase *a* form was determined as the difference between corrected AMP + caffeine activity and activity of the phosphorylase *ab* hybrid.

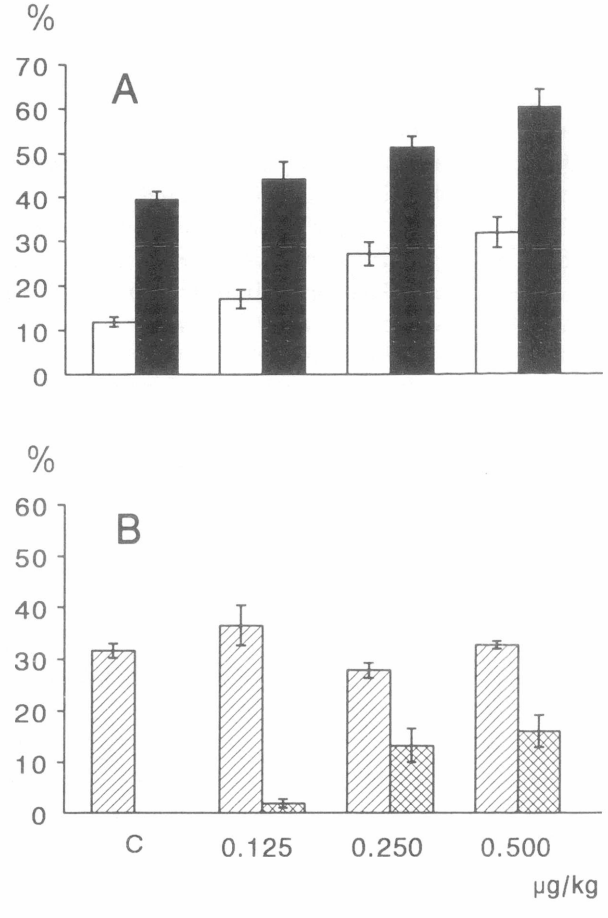

Fig. 1
Formation of the phosphorylase *ab* hybrid and phosphorylase *a* in rat heart 15 s after i.v. isoproterenol administration. Values are expressed in per cent of total phosphorylase.
A. Phosphorylase activity without AMP (open columns) and phosphorylase activity with 1 mM AMP + 5 mM caffeine (full columns).
B. Calculated amounts of the phosphorylase *ab* hybrid (hatched columns) and phosphorylase *a* form (cross-hatched columns). Each column represents mean ± S.E.M. of 6–8 experiments.

Results

Figs 1A and 2A show phosphorylase activity estimated without AMP and with AMP + caffeine as % of total phosphorylase estimated with AMP. The activities of particular enzymes are expressed as % of total phosphorylase activity because this did not change throughout the experiment. In the control rat hearts, the phosphorylase activity estimated without AMP was 11 % of the total activity. Caffeine, which blocks the phosphorylase *b* activity, in the presence of AMP significantly decreased phosphorylase activity. The phosphorylase activity in the presence of caffeine, exhibits only 38 % of the total value.

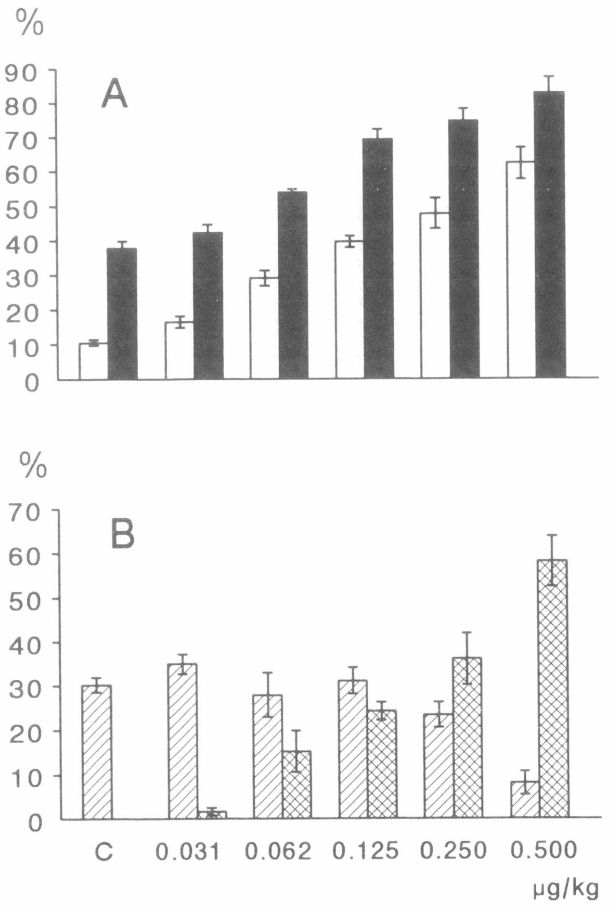

Fig. 2
Formation of the phosphorylase *ab* hybrid and phosphorylase *a* in rat heart 40 s after i.v. isoproterenol administration. Values are expressed in per cent of total phosphorylase. A. Phosphorylase activity without AMP (open columns) and phosphorylase activity with 1 mM AMP + 5 mM caffeine (full columns). B. Calculated amounts of the phosphorylase *ab* hybrid (hatched columns) and phosphorylase *a* form (cross-hatched columns). Each column represents mean ± S.E.M. of 6–8 experiments.

Fig. 1A shows both phosphorylase activities 15 s after isoproterenol stimulation. A dose-dependent increase of phosphorylase activity from total phosphorylase determined without AMP was present 40 s after isoproterenol administration (Fig. 2A). The maximal stimulation of phosphorylase activity estimated without AMP was 62 % of the total phosphorylase activity. An increased dose of administered isoproterenol, however, decreased the difference between phosphorylase activities without AMP and with AMP + caffeine, suggesting that the amounts of the produced phosphorylase *a* form was increased.

Figs 1B and 2B show the amount of the phosphorylase *ab* hybrid and phosphorylase *a* calculated according to Vereb *et al.* (1986).

In control rat hearts, no phosphorylase *a* was present and the phosphorylase *ab* hybrid was 30 % of the total phosphorylase activity. Phosphorylase *ab* hybrid was increased up to 36.5 % of total phosphorylase after isoproterenol stimulation. Longer time (40 s) and higher stimulation (dose of isoproterenol 0.25–0.5 $\mu g.kg^{-1}$) was needed for the transformation of phosphorylase *b* to phosphorylase *a*. Under these conditions the phosphorylase activity *a* increased in a dose-dependent manner and reached maximal level corresponding to 58 % of total phosphorylase activity. The phosphorylase *ab* hybrid was always present even at the maximal transformation of phosphorylase *b* into phosphorylase *a* form.

Discussion

Conversion of glycogen phosphorylase *b* to *a* is now generally accepted to be the result of a cascade of phosphorylation reactions (Namm and Mayer 1981).

In spite of the fact that transformation of phosphorylase *b* to its *a* form is considered as the simplest example of covalent regulation (Walsh *et al.* 1991), which is also influenced allosterically (Dombrádi 1981), this conversion is now known to be a two-step process. The above mentioned process proceeds *via* intermediary formation of the phospho-dephospho phosphorylase *ab* hybrid.

The formation of phosphorylase *ab* hybrid was previously demonstrated to occur under *in vitro* conditions in rabbit skeletal muscle (Hurd *et al.* 1966, Bot *et al.* 1974). The phosphorylase *ab* hybrid was detected using both the analysis of kinetics of enzyme properties and ^{32}P incorporation into phosphorylase. "Flash" glycogen phosphorylase activation of the protein-glycogen complex, isolated from rabbit skeletal muscle indicated that the phosphorylase *ab* hybrid may also be formed in muscle *in vivo* (Heilmeyer *et al.* 1970). Gergely *et al.* (1974) confirmed the formation of the phosphorylase *ab* hybrid *in vivo* in rabbit skeletal muscle as a response to both hormonal and neural signals. The phosphorylase *ab* hybrid was found to exist *in vivo* in fish muscle (Schmidt and Wegener 1990), in fat body of insects (van Marrewijk *et al.* 1988, Gäde 1991) and in lungworm (Kamp and Winnemöller 1992).

The phosphorylase *ab* hybrid in the rat heart was shown only by Vereb *et al.* (1986) who demonstrated it under *in vitro* conditions and in the myocardium obtained from isolated hearts prepared and perfused according to Langendorf.

In our experiments the formation and further transformation of the phosphorylase *ab* hybrid was shown *in vivo*, in rat hearts obtained from artificially respirated animals.

Quantitative calculation of the phosphorylase *ab* hybrid of the rat myocardium phosphorylase is based on the following facts: a) activity of the phosphorylase *ab* hybrid measured in the presence of 16 mM glucose-1-phosphate is doubled with 1 mM AMP, even in the presence of 5 mM caffeine and b) caffeine blocks the activation of phosphorylase *b* by AMP (Vereb *et al.* 1986).

The other fact, which was taken into consideration, was that the rat heart phosphorylase differs from the phosphorylases occurring in skeletal muscles. The results of Berndt and Rösen (1984) suggest the existence of two native forms of phosphorylase *b* in the rat heart. The heart specific form of phosphorylase has different enzymatic properties than the skeletal muscle enzyme. The occurrence of the heart specific component in the rat heart phosphorylase represented about 20 % activity of the *b* form in the presence of caffeine and AMP as was shown by Vereb *et al.* (1986).

In our experiments it was mainly the partially phosphorylated phosphorylase which was revealed immediately (15 s) after the administration of small isoproterenol doses. Later, after higher isoproterenol doses, the phosphorylase *ab* hybrid form which was present at all degrees of stimulation, was changed to phosphorylase *a*. In the control rat hearts only the phosphorylase *ab* hybrid was determined.

By contrast, in rabbit skeletal muscles only the phosphorylase *ab* hybrid form was estimated after hormonal and electrical stimulation (Gergely *et al.* 1974). On the other hand, the reaction *in vitro*, was readily completed with the full formation of phosphorylase *a* (Bot *et al.* 1974).

Similar results were obtained by Vereb *et al.* (1986) in the rat heart during *in vitro* phosphorylation reactions. The formation of the phosphorylase *ab* hybrid was transient. However, in perfused isoproterenol-stimulated hearts both phosphorylase *ab* hybrid and *a* were formed.

Both in anaesthetized and exercised fish white skeletal muscle all three forms of phosphorylase were determined (Schmidt and Wegener 1990). Three interconvertible forms of phosphorylase exist simultaneously in the fat body of insects both at rest and after peptide stimulation (Gäde 1991). Interesting are thre results of Kamp and Winnemöller (1992) who registered predominantly the existence of the phosphorylase *ab* hybrid not only *in vitro* but also *in vivo*. The results did not confirm any phylogenetic differences in comparison to the rat.

To study the stimulatory effect on phosphorylase interconversion only one dose or a single stimulus were used in most of the above mentioned experiments. A better insight into the transformation of each of the three forms of phosphorylase will show the phosphorylase stimulation in a dose-dependent manner, as was performed in our study.

Our results completely confirmed that the standard utilization of −AMP/+AMP ratio does not provide full information as to whether it is phosphorylase *ab* hybrid or phosphorylase *a* which is produced. The −AMP/+AMP ratio reflects rather the ratio of phosphorylated subunits than the percentage proportion of activated phosphorylase molecule.

We speculated that the formation of the phosphorylase *ab* hybrid in the rat heart *in vivo* together with phosphorylase *a* may lead to a faster, more sensitive and more flexible response of the phosphorylase system to metabolic stimulation. The simultaneous existence of the *ab* hybrid and *a* form of phosphorylase may increase the regulatory function of phosphorylase *in vivo* which is very important in heart metabolism.

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Reprint Requests

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