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Species-dependent differences in the properties of particulate cyclic nucleotide phosphodiesterase from rat and rabbit ventricular myocardium

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Abstract—The ability of cyclic nucleotide phosphodiesterases (PDEs) to hydrolyse cyclic (c)AMP in rat and rabbit ventricular myocardium has been compared. The PDE activity of rabbit, but not rat, cardiac homogenate and supernatant fraction was potentiated by Ca^{2+}/calmodulin and attenuated by cGMP. Both rabbit and rat ventricular myocardium were shown to have a membrane bound PDE. However, rabbit membrane-bound PDE was inhibited by cGMP and low concentrations of milrinone (IC50 2·7 μM). In contrast, rat membrane-bound PDE was not inhibited by either cGMP or low concentrations of milrinone (IC50 19 μM), but it was potently inhibited by rolipram (IC50 2·2 μM). Thus, in rabbit the particulate PDE is milrinone sensitive (PDE III) whilst in rat it is the rolipram sensitive (PDE IV) isoenzyme. There are clearly species differences in the intracellular localization and relative activities of PDE isoenzymes in cardiac tissue. This may explain the species differences already found in the activity of selective PDE isoenzyme inhibitors as inotropic agents.

Cyclic nucleotide phosphodiesterases (PDEs) exist in multiple molecular forms (Beavo 1988), and at least four different subtypes have been identified in guinea-pig, rat and human ventricular myocardium (Reeves et al 1987; Nicholson et al 1989b). The PDE isoenzymes identified in cardiac tissue and termed PDE I–IV by Reeves et al (1987) can be characterized according to the Beavo classification as follows: PDE I is stimulated by Ca^{2+}/calmodulin; PDE II is stimulated by cyclic (c) GMP; PDE III has a low Km for cAMP and is inhibited by cGMP; PDE IV has a low Km for cAMP, and in contrast to PDE III has no known regulator, but it is selectively inhibited by Ro 20-1724 and rolipram. PDE III has been suggested to be particularly important in regulating cardiac contractility, since a number of new positive inotropic agents produce selective and potent inhibition of this isoenzyme (Weishaar et al 1987; Kithas et al 1988). These compounds, although active in a number of species including dog and rabbit do not elicit inotropic responses in rat ventricular myocardium (Weishaar et al 1987). It has been suggested that inhibition of a membrane-bound PDE III activity is responsible for the inotropic activity of PDE inhibitors and that the inability of compounds such as imazodan to increase cardiac contractility in the rat may be due to PDE III being present solely in the cardiac cell cytosol in this species (Weishaar et al 1987). The aim of the present experiments was to analyse further this suggestion by comparing the distribution and properties of PDE isoenzymes in rat and rabbit ventricular myocardium.

Materials and methods

All procedures during enzyme preparation were performed at 4°C. Fresh cardiac ventricular tissue (4–5 g) from male Wistar rats and male New Zealand White rabbits was homogenized (Polytron; PT20 probe, two bursts at speed settings 5 and 7 each of 10 s duration) in 10 volumes of buffer (pH 6·5) containing (mmol L^{-1}): Bistris 20, dithiothreitol 1, benzamidine 2, ethylenediaminetetraacetic acid 2, Na-acetate 50 and phenylmethanesulphonyl fluoride 0·1. The homogenate (H) was filtered through two layers of cheesecloth and centrifuged at 12000 *g* for 10 min (Damon IEC M60 ultracentrifuge; rotor 410) and the pellet (P) and supernatant (S) fractions were used as source of particulate- and soluble-PDE, respectively. The pellet fraction was washed with buffer (40–50 mL) to remove residual soluble-PDE. Phosphodiesterase activity was measured according to the method described by Shahid & Rodger (1989), using cAMP (1 μM) as substrate. Concentrations of milrinone and rolipram producing 50% inhibition of PDE activity (IC50 values) are given as geometric mean with 95% confidence limits from 4–5 separate experiments.

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Results

The results are shown in Table 1. It is clear that, at a substrate concentration of 1 μM PDE activity was much higher in rabbit ventricle than in the rat ventricle. However, the relative distribution of PDE activities in the two species was similar (50–65% in S and 30–35% in P). The sensitivities of rat H, S and P fractions to the regulators Ca^{2+}/calmodulin and cGMP were markedly different from those of the rabbit in which the H, S and P fractions were inhibited (36 ± 4; 27 ± 3 and 49 ± 6%, respectively) by a low concentration of cGMP whilst only the H and S fractions were stimulated by Ca^{2+}/calmodulin (33 ± 7 and 70 ± 13%, respectively). In contrast, the rat PDE fractions were not significantly affected by either Ca^{2+}/calmodulin or cGMP.

The effects of the high-affinity cAMP PDE inhibitors, milrinone and rolipram were examined on rat and rabbit particulate PDE. Whereas milrinone was a more effective inhibitor of rabbit P fraction (IC50: 2·7 (1·4–5·1) μM) than rat P fraction (IC50: 19 (11–31) μM), rolipram was a much more potent inhibitor of rat P fraction (IC50: 2·2 (1·3–3·9) μM) than rabbit P fraction (IC50: > 250 μM, 43 ± 5% inhibition at 250 μM).

Discussion

The present study has shown that there are few differences in the relative distribution of the cytosolic and membrane-bound cAMP hydrolytic activities of PDE isoenzymes in rat and rabbit cardiac ventricle. However, there are significant differences in the total PDE activity, as well as in the sensitivity of cytosolic and membrane-bound PDEs to regulatory and inhibitory substances. The levels of absolute PDE activity observed in rabbit and rat ventricle in the present study are in agreement with the results of Kithas et al (1988) and Kakiuchi et al (1975), respectively. The lack of effect of Ca^{2+}/calmodulin or cGMP on rat H and S fractions suggests that the relative activities of the Ca^{2+}/calmodulin dependent or the cGMP stimulated PDE isoenzymes differ in rat and rabbit ventricle. The stimulation of PDE activity by exogenous Ca^{2+}/calmodulin in rabbit myocardium indicates that PDE I is active at low substrate concentrations in this species. The lack of activation of rat -H and -S fractions by Ca^{2+}/calmodulin indicates that either PDE I, which is present in rat myocardium as a cAMP high affinity isoenzyme (Nicholson et al 1989b), represents a smaller proportion of the total PDE activity in rat, compared with rabbit ventricle (at least at a substrate concentration of 1 μM cAMP), or that there may

Table 1. cAMP-PDE activities in rat and rabbit cardiac ventricle.

	Rat			Rabbit		
	Specific activity (pmol min^{-1} $(mg\ prot)^{-1}$)			Specific activity (pmol min^{-1} $(mg\ prot)^{-1}$)		
Fraction	Control	+Ca/Cm	+cGMP	Control	+Ca/Cm	+cGMP
H	23·5±1·6	26·4±2·2	26·7±2·0	159±33	217±44*	85±17*
S	79·8±15	79·9±12·3	77·7±9·5	383±72	662±106**	217±40*
P	10·5±2	10·8±2·4	10·4±2·1	107±44	109±45	46±11*

H-cardiac homogenates; S-supenatant fraction; P-pellet fraction (preparation see text). [Ca] 20 μM, [Calmodulin; Cm] 1·5 $\mu g\ mL^{-1}$; [cGMP] 1 μM; * $P<0·05$, ** $P<0·01$ (Paired Student's t-test) compared with corresponding control value. Each value is the mean ± s.e.m. of 5–6 preparations.

already be sufficient endogenous Ca^{2+}/calmodulin in rat ventricle to fully activate the PDE I present. In rabbit H, S and P fractions, cGMP inhibited cAMP hydrolysis. This is consistent with the high activity of cGMP inhibited cAMP PDE in this species (Kithas et al 1988). It is difficult to interpret the lack of effect of cGMP on rat H and S fractions, since whilst cGMP inhibits PDE III activity, the cAMP hydrolytic activity of PDE II is stimulated. Thus, the effect of cGMP on cAMP hydrolysis will depend upon the relative activities of PDE II and PDE III in each tissue. It is possible that a net effect of no change in cAMP hydrolysis may be observed if both PDE II and PDE III are present in sufficient quantities. Certainly both these isoenzymes are present in the rat myocardium (Nicholson et al 1989b).

The most important finding of this study was the differing properties of membrane-bound PDE in rat and rabbit ventricle. The inhibition of the rabbit P fraction by cGMP and milrinone and the weak attenuation by rolipram, are in agreement with the results reported by Kithas et al (1988) and consistent with the suggestion that the membrane-bound PDE in rabbit ventricle is PDE III. In contrast, the lack of effect of cGMP, the relatively weak effect of milrinone and the potent inhibition by rolipram, of rat P fraction, suggest this activity to be PDE IV-like in character.

The finding that there are species-dependent differences in membrane-bound PDE activity is supported by the work of Simmons & Hartzell (1988) who showed that the membrane-bound cAMP PDE activity in frog ventricle was stimulated by cGMP, indicating that it was PDE II-like. The rat P results obtained in the present study extend the work of Ahluwalia et al (1984). This group established the presence of membrane-bound PDE in rat heart but did not determine regulatory characteristics or inhibitor sensitivity. However, these data conflict with the results of Weishaar et al (1987) who reported the absence of any membrane-bound PDE activity in rat ventricle. The precise reasons for this discrepancy are unclear but they could be related to differences in the method of enzyme preparation; the sonication of cardiac homogenate to dislodge membrane bound-PDE was not employed in our protocol. In contrast to other species such as rabbit and dog (see introduction), selective PDE III inhibitors do not elicit inotropic responses in the rat. This has been attributed to the lack of membrane-bound PDE III in rat heart (Weishaar et al 1987). It has been suggested that PDE III inhibitors owe their inotropic activity to inhibition of a particulate PDE III isoenzyme (Weishaar et al 1987; Kithas et al 1988). Our data suggest that there is a PDE IV membrane-bound PDE activity in rat heart. However, like the selective PDE III inhibitors, PDE IV inhibitors, such as rolipram, also fail to mediate direct inotropic responses in rat heart (Weishaar et al 1987; Nicholson et al 1989a). Some inhibitors of cyclic nucleotide phosphodiesterase do, however, increase contractility in rat myocardial preparations 3-isobutyl-1-methylxanthine, a non-selective PDE inhibitor, and Org 30029 (*N*-hydroxy-5,6-dimethoxybenzo [b] thiophene-2 carboximid amide HCl), a dual inhibitor of PDE III and IV, both increase developed tension in rat isolated papillary muscles (Nicholson et al 1989a). Thus, to produce positive inotropism in rat heart it is at least necessary to inhibit both PDE III and PDE IV. The results of the present study throw doubt on the hypothesis that inhibition of membrane-bound PDE, is solely responsible for the inotropic activity of PDE inhibitors in all species. In rat heart, inotropic activity does not appear to be correlated to inhibition of a particulate PDE-isoenzyme. Differences either in the precise intracellular distribution of PDE III and PDE IV isoenzymes or in the relative activities of the PDE isoenzymes in rat and rabbit ventricle may be responsible for the lack of inotropic activity of selective PDE III and PDE IV inhibitors in rat ventricular myocardium.

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