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SHORT COMMUNICATIONS

Comparison of β-adrenergic receptors and the adenylate cyclase system with muscarinic receptors and guanylate cyclase activities in the heart of spontaneously hypertensive rats

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Recent studies indicated that the number of β-adrenergic [1] and secretin-VIP* [2] receptors coupled to adenylate cyclase, and the activity of cyclic GMP-dependent protein kinase [3] is depressed in the enlarged heart of spontaneously hypertensive (SH) rats. In the same organ, on the other hand, the number and affinity of muscarinic cholinergic receptors and the activity of guanylate cyclases has not been fully established.

In the normal rat heart, it is known that a mutual antagonism exists between the activation of β-adrenergic and muscarinic cholinergic receptors, in terms of their abilities to regulate the contractile responses and the intracellular levels of cyclic AMP and cyclic GMP. In addition, each of the cyclic nucleotides and calcium appears capable of modifying the metabolism and biochemical actions of the other two. Epinephrine stimulates the formation of cyclic AMP and the phosphorylation of proteins established as substrates for the cAMP-dependent protein kinase in the perfused rat heart [4] and this may result in the activation of calcium pumps and in the contractile response [5]. Acetylcholine-induced increases in cyclic GMP appear to correlate with decreased contractile force of the heart and inhibition of cathecholamine stimulated increase in cyclic AMP levels [6–11]. The 8-bromo analog of cyclic GMP produces also a negative inotropic effect in rat atrial muscle [12]. These observations suggest that cyclic GMP may be involved in mediating the negative inotropic action of acetylcholine on the heart as well as its ability to antagonize the action of β-adrenergic catecholamines. The aim of the present study was to compare muscarinic binding sites and the activity of soluble and particulate guanylate cyclases to β-adrenoreceptors and adenylate cyclase activity in the heart of spontaneously hypertensive (SH) rats and normotensive control animals (WKY).

Experiments were conducted on male SH rats of the Okamoto strain, 18 weeks of age, and age-matched normotensive Wistar-Kyoto (WKY) rats. The systolic blood pressure was 198 ± 7 mm Hg in SH rats and 123 ± 5 mm Hg in WKY animals (mean ± SEM, $n = 5$ in both groups). These animals were killed by decapitation and exsanguinated. The cardiac weight of SH rats (993 ± 20 mg) was significantly higher than that of WKY animals (860 ± 20 mg). Each heart was dissected out, rinsed with 0.154 M NaCl, and homogenized (5% w/v homogenate) in a buffer consisting of 20 mM Tris–HCl (pH 7.4), 2 mM dithioerythritol, and 5 mM $MgCl_2$ at 4°. After filtration through two layers of medical gauze, the homogenate was centrifuged at 520 *g* for 10 min. The crude particulate extract was treated as previously described [2] and membranes obtained were tested for β-adrenergic and muscarinic receptors and for adenylate cyclase activity (*vide infra*). The supernatant was further centrifuged at 100 000 *g* for 60 min. The activity of soluble guanylate cyclase was tested in the 100 000 *g* supernatant and that of particulate guanylate cyclase was tested in the final pellet washed with 25 mM Tris–HCl buffer (pH 7.5), 1 mM dithioerythritol, and resuspended in the same buffer.

β-Adrenergic receptors in heart membranes were evaluated by incubating for 60 min at 25° 90–110 μg protein with increasing concentrations or a fixed 0.2 nM concentration of the antagonist [^{125}I]hydroxybenzylpindolol ([^{125}I]HYP; specific activity 2200 Ci/mmole, obtained from New England Nuclear Company, Dreieich, Germany) in 120 μl of 20 mM Tris–maleate, 5 mM $MgCl_2$, 1 mM dithioerythritol, 0.1 mg/ml bacitracin, 0.1 mg/ml ascorbic acid, and 1% bovine serum albumin, pH 7.0. The binding capacity was tested without or with 1 μM added (±) pindolol. Membrane bound radioactivity was separated from free radioactivity by filtration through glass fiber filters GF/C (Whatman, Maidstone, England) and washed three times with ice-cold buffer. Specific binding was defined as maximal binding less the binding in the presence of pindolol.

For muscarinic receptor determination, heart membranes (90–110 μg protein) were incubated for 60 min at 25° with increasing concentrations or a fixed 0.5 nM concentration of the antagonist 3-[^{3}H] quinuclidinyl benzilate ([^{3}H]QNB, specific radioactivity 16 Ci/mmole, obtained from the Radiochemical Centre, Amersham, England) in 1.20 ml of 20 mM Tris–HCl, 5 mM $MgCl_2$, and 1 mM dithioerythritol, pH 7.0, in the absence or presence of 1 μM atropine. Membrane bound radioactivity was processed as above.

Adenylate cyclase activity in cardiac membranes was determined as previously described [2] using the Salomon *et al.* procedure [13]. Guanylate cyclase activity was determined by incubating 40 μg of soluble protein or 25 μg of particulate protein in 60 μl of 50 mM Tris–HCl, 1 mM isobutylmethylxanthine, 1 mM cyclic GMP, 1 mM dithioerythritol, 0.1 mg/ml bovine serum albumin, 10 mM creatine phosphate, 0.2 mg/ml creatine kinase (140 units/mg), 0.4 mM [α-^{32}P]-GTP (2×10^6 cpm), and 2 mM $MnCl_2$, pH 7.6. The reaction, initiated by the addition of the enzyme, was conducted for 10 min at 37° and the labelled cyclic GMP formed was purified according to White and Karr [14].

Cardiac membranes from WKY and SH rats bound QNB with high affinity at equilibrium (app K_D of 0.5–0.6 nM). The muscarinic receptors were comparable in both substrains, based on [^{3}H]-QNB binding (Fig. 1, left panel; and Table 1), and the relative potency of dexetimide and levitimide [9], and of a variety of agonists to inhibit [^{3}H]-QNB binding used at a fixed 0.5 nM concentration (data not shown).

By contrast, and in line with previous data from Limas and Limas [1], the maximum number of β-adrenoreceptors for the antagonist hydroxybenzylpindolol was reduced by 66 per cent in cardiac membranes from hypertensive SH rats when compared to that in normotensive WKY controls (Fig. 1, right panel; and Table 1) without a change in the affinity of the corresponding binding sites for the antagonist (Fig. 1) and for a variety of β agonists (data not shown).

The activity of soluble and particulate guanylate cyclases, when determined under optimal manganese concentration, was comparable to values reported by Kimura *et al.* [15] and Katsuki *et al.* [16], and was similar in hypertensive and

* *Abbreviations used:* HYP, hydroxybenzylpindolol, QNB, 3-quinuclidinyl benzilate; VIP, vasoactive intestinal peptide; Gpp(NH)p, guanosine 5′-*O*-(2-3-imido)triphosphate; SH, spontaneously hypertensive; WKY, Wistar Kyoto.

Table 1. Dissociation constant (K_d) of 3-[^{3}H] quinuclidinyl benzilate and [^{125}I] hydroxybenzylpindolol and maximal number of binding sites in heart membranes from 18-week-old WKY and spontaneously hypertensive male rats

Parameter	3-[^{3}H]-QNB binding		[^{125}I]-HYP binding	
	WKY rats	SH rats	WKY rats	SH rats
K_d (nM)	0.55 ± 0.02	0.54 ± 0.02	0.13 ± 0.01	0.12 ± 0.02
Binding sites (fmoles × mg prot^{-1})	250 ± 30	240 ± 35	38.8 ± 4.0	13.2 ± 1.5*

Both parameters were determined at equilibrium from Scatchard plot analysis as described in Fig. 1. Results are the means ± SEM from 5 animals.
* Values in SH rats significantly lower than in WKY rats ($P < 0.05$).

control rats (Table 2). Oleic acid and Triton X-100 stimulated significantly the particulate enzyme but not the soluble form and here also there was no difference between SH and WKY rats.

By contrast, adenylate cyclase activity was altered in the heart of SH rats (Table 3). As previously shown in 14 week-old animals [2] and confirmed here in 18-week-old rats, adenylate cyclase alterations were limited to the receptor side of the membrane since basal, GTP-, Gpp(NH)p, and NaF-stimulated adenylate cyclase activities were identical in both substrains of rats whereas the isoproterenol-stimulated enzyme was half as active in SH rats as in normotensive animals.

Our results indicate that the number and efficacy of muscarinic receptors were normal in the heart of 18 weeks old spontaneously hypertensive (SH) rats. The basal and stimulated activities of soluble and particulate guanylate cyclases were also normal. In line with the latter results, Amer *et al.* [17] observed no change in "basal guanylate cyclase activity" in the heart of SH rats but cyclic GMP levels were lower than in control heart, due possibly to a higher activity of low K_m cyclic GMP phosphodiesterase. These data were, however, not documented.

We observed that the number of β-adrenergic receptors and the concurrent activity of isoproterenol-stimulated adenylate cyclase were reduced with no apparent alteration in adenylate cyclase subunits located at the inner face of heart plasmalemma membranes. Our results contrast with those of Bhalla *et al.* [18] and Bhalla and Ashley [19] who inferred that the number of adenylate cyclase molecules was decreased and/or the kinetic properties of the enzyme were altered in the heart of SH rats rather than the number of β-adrenergic receptors, based on a decreased sensitivity to catecholamines of adenylate cyclase activation curves. By contrast, and in line with our data, Limas and Limas [1] found that the number of β-adrenergic receptors was decreased by 30–40 per cent in SH rats, without alteration in their sensitivity to catecholamines.

It is concluded that in SH rats the hea t system involving cyclic AMP is much more impaired than that controlling cyclic GMP. The myocardial hypertrophy in SH rat thus differs in several respects from that observed in four other models of heart hypertrophy: (1) pressure overload produced by constriction of the abdominal aorta induces a 40 per cent increase in the number of β-receptors in the rat heart within 4 weeks after the operation [20]; (2) hyperthyroidism induces a marked increase in the number of β-receptor binding sites [21] and a significant decrease in

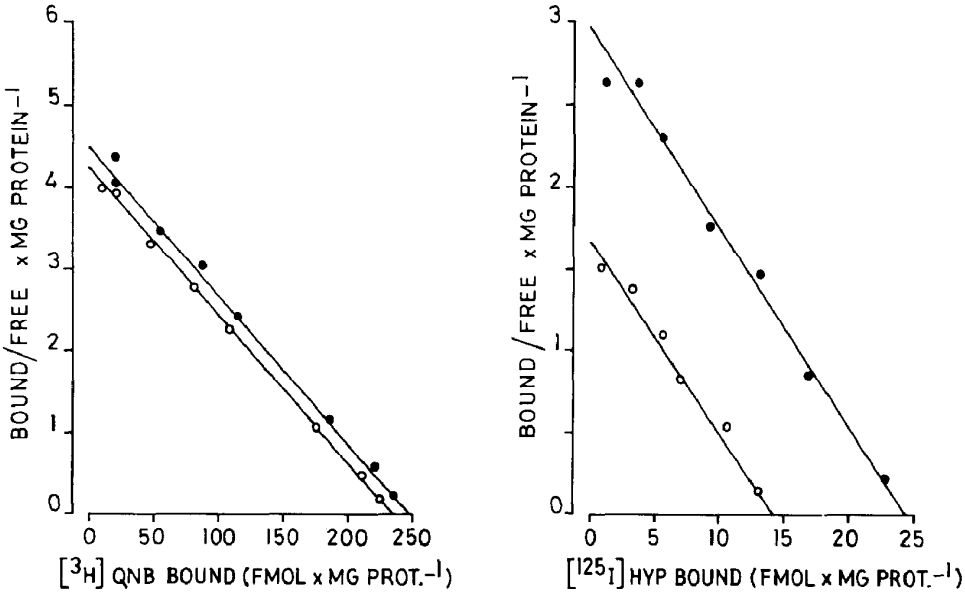

Fig. 1. Scatchard plots of specific binding of 3-[^{3}H]quinuclidinyl benzilate (left panel) and [^{125}I]hydroxybenzylpindolol (right panel) to cardiac membranes from 18-week-old WKY (○—○) and SH (●—●) male rats. Typical plots with heart membranes from one animal in each group are shown. The negative reciprocal of the slope provides an estimate of the equilibrium dissociation constant (K_d) for the interaction of the radioactive ligand with binding sites and the intercept in the abcissa indicates the maximal number of binding sites. All Scatchard plots yielded straight lines, suggesting uniform populations of receptors with high affinity for the muscarinic and β-adrenergic antagonists tested.

Table 2. Basal and stimulated activities of soluble and particulate guanylate cyclases in heart from 18-week-old WKY and spontaneously hypertensive male rats

Addition	Soluble enzyme		Particulate enzyme	
	WKY rats	SH rats	WKY rats	SH rats
None (basal value)	10.7 ± 0.5	12.2 ± 0.4	6.6 ± 0.3	7.0 ± 0.4
Oleic Acid 1 mM	10.3 ± 0.3	10.7 ± 0.2	8.5 ± 0.6*	8.4 ± 0.5*
Triton X-100 1%	10.6 ± 0.5	10.4 ± 0.2	22.1 ± 1.3*	22.8 ± 2.4*

Data were expressed as pmoles cyclic GMP formed × min^{-1} × mg $protein^{-1}$ (mean ± SEM from 5 animals).

* Stimulated values significantly higher than basal values ($P < 0.05$). There were no differences between hypertensive (SH) and normotensive (WKY) rats.

Table 3. Adenylate cyclase activity in heart membranes from 18-week-old WKY and spontaneously hypertensive male rats.

Addition	WKY rats	SH rats
None	45 ± 5	43 ± 5
NaF 10 mM	493 ± 50	450 ± 48
Gpp(NH)p 10 μM	142 ± 15	128 ± 16
GTP 10 μM	54 ± 7	50 ± 7
Isoproterenol 0.1 mM + GTP 10 μM	220 ± 20	135 ± 15*

Data expressed as pmols cyclic AMP formed × min^{-1} × mg $protein^{-1}$. The results are the mean ± SEM from 5 animals.

* Values in SH rats significantly lower than in WKY rats ($P < 0.05$).

the number of muscarinic receptors [22]; (3) chronic isoproterenol treatment elevates the levels of cyclic GMP which may be due to decreased activity of the cyclic GMP phosphodiesterase, and elevates the stimulatory modulator of cyclic GMP-dependent protein kinase which may increase expression of cyclic GMP effects [23]. This contrasts with a decreased number of β-adrenergic receptors and a decreased response of adenylate cyclase to both catecholamines and sodium fluoride [24]; (4) in Goldblatt rats with renal hypertension, the heart adenylate cyclase system appears unchanged when compared to sham-operated animals [2]. It is thus tempting to conclude that the present heart anomalies reflected a part of the syndrome of congenital hypertension in rat and not the mere consequence of cardiac hypertrophy.

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Department of Biochemistry and Nutrition
Medical School, Université Libre de Bruxelles
Bld. de Waterloo 115, *B*-1000 *Brussels, Belgium*

PATRICK ROBBERECHT
JACQUES WINAND
PIERRE CHATELAIN
PIOTR POLOCZEK
JEAN-CLAUDE CAMUS
PHILIPPE DE NEEF
JEAN CHRISTOPHE

* To whom correspondence should be addressed.

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